NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF A NONDECOLORIZED WHOLE LEAF EXTRACT OF ALOE BARBADENSIS MILLER (ALOE VERA) IN F344/N RATS AND B6C3F$_1$ MICE

(DRINKING WATER STUDY)

Scheduled Peer Review Date: April 5, 2011

NTP TR 577

NIH Publication No. 11-5919

National Toxicology Program

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*) or in hardcopy upon request from the NTP Central Data Management group at *cdm@niehs.nih.gov* or (919) 541-3419.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF A NONDECOLORIZED WHOLE LEAF EXTRACT OF ALOE BARBADENSIS MILLER (ALOE VERA) IN F344/N RATS AND B6C3F$_1$ MICE

(DRINKING WATER STUDY)

Scheduled Peer Review Date: April 5, 2011

NTP TR 577

NIH Publication No. 11-5919

National Toxicology Program

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

CONTRIBUTORS

This study on Aloe vera was conducted at the Food and Drug Administration's (FDA) National Center for Toxicological Research (NCTR) under an interagency agreement between the FDA and the National Institute of Environmental Health Sciences (NIEHS). The study was designed and monitored by a Toxicology Study Selection and Review Committee composed of representatives from the NCTR and other FDA product centers, NIEHS, and other ad hoc members from other government agencies and academia. The interagency agreement was designed to use the staff and facilities of the NCTR in the testing of FDA priority chemicals and to provide FDA scientists and regulatory policy makers information for hazard identification and risk assessment.

National Center for Toxicological Research, Food and Drug Administration

Conducted study, evaluated and interpreted results and pathology findings, and reported findings, and prepared the study report

M.D. Boudreau, Ph.D., Study Scientist
F.A. Beland, Ph.D.
J.A. Nichols, B.S.
M. Pogribna, M.D., M.P.H.

Conducted microbiology surveillance and diagnostics

R.D. Wagner, Ph.D.
D.D. Paine, B.S.
C. Summage-West, B.S.
R.S. Steele, B.S.
L.M. Sims, B.S.

Conducted dose certifications and chemical analyses

P.H. Siitonen, B.S.
B. Brown, B.S.
C.R. Cozart, B.S.
T.C. Schmitt, B.S.

Conducted statistical analyses

R.P. Felton, M.S.
B.T. Thorn, M.S.

Conducted quality assurance audits

J.M. Fowler, B.S.
Y.E. Whiteside, B.S.

Prepared Technical Report

R.L. Stingley, Ph.D., Project Leader
S.C. Matson, Ph.D.
P.C. Howard, Ph.D.
C.C. Weis, B.S.

National Institute of Environmental Health Sciences

Reviewed and evaluated the technical report, interpreted results and pathology findings

N.J. Walker, Ph.D.
D.E. Malarkey, D.V.M., Ph.D.
P.M. Foster, Ph.D.
C.J. Alden, Ph.D.
G.S. Travlos, D.V.M.
G.E. Kissling, Ph.D.
J.K. Dunnick, Ph.D.
B.J. Collins, M.S.P.H.
K.L. Witt, Ph.D.

Bionetics Corporation
Prepared animal feed, dosed water solutions, and provided animal care

J. Carson, B.S.
L. Conner
F. Lewis
A. Matson, B.S.

Toxicologic Pathology Associates
Evaluated pathology findings

P.W. Mellick, D.V.M., Ph.D.,
Study Pathologist (Rats)
G.R. Olson, D.V.M., Ph.D.,
Study Pathologist (Mice)
A. Warbritton
L. Wiley, B.S.

Experimental Pathology Laboratories, Inc.
Provided pathology review

R.A. Miller, D.V.M., Ph.D. *(Quality assessment review (October 6-10, 2008))*
E. Terence Adams, D.V.M., Ph.D. *(Pathology Working Group Coordinator)*
A.E. Brix, D.V.M., Ph.D.

Z-Tech Corporation
Provided software systems development and data entry

K.A. Carroll
S. Goldman

NTP Pathology Working Group
Evaluated slides and prepared pathology reports (November 2008)

E. Terence Adams, D.V.M., Ph.D., Coordinator
Experimental Pathology Laboratories, Inc.
G. Flake, M.D.
National Institute of Environmental Health Sciences
J.R. Latendresse, D.V.M., Ph.D.
Toxicologic Pathology Associates
D.E. Malarkey, D.V.M., Ph.D.
National Institute of Environmental Health Sciences
R.R. Maronpot, D.V.M., M.S., M.P.H.
Experimental Pathology Laboratories, Inc.
P.W. Mellick, D.V.M., Ph.D.
Toxicologic Pathology Associates
Study Pathologist (Rats)
R.A. Miller, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
G.R. Olson, D.V.M., Ph.D.
Toxicologic Pathology Associates
Study Pathologist (Mouse)
S. Francke-Carroll, D.V.M., Ph.D.
Center for Food Safety and Nutrition (Observer)

NIEHS/FDA Interagency Agreement Project Officers

P.C. Howard, Ph.D.
National Center for Toxicological Research
W.T. Allaben, Ph.D.
National Center for Toxicological Research
N.J. Walker, Ph.D.
National Institute of Environmental Health Sciences
J.R. Bucher, Ph.D.
National Institute of Environmental Health Sciences

CONTENTS

ABSTRACT

Aloe barbadensis Miller, Aloe vera, has enjoyed a long history of lay acceptance as an herbal remedy and is perhaps the most popular herbal remedy in use today. In recent times, the oral consumption of Aloe vera has been promoted as a prophylaxis and treatment to alleviate a variety of unrelated systemic conditions. The National Cancer Institute nominated Aloe vera for study under the National Toxicology Program, because of its widespread human exposure and because components in Aloe vera may possess tumor-promoting activities. Male and female F344/N rats and B6C3F$_1$ mice were exposed to freeze dried (max. 6% moisture) and gamma-irradiated extracts of Aloe vera plant leaves in drinking water for 14 days, 13 weeks, or 2 years.

14-DAY STUDY IN RATS

Groups of four male and four female F344/N rats were administered Aloe vera gel, Aloe vera nondecolorized whole leaf, or Aloe vera decolorized whole leaf extracts in drinking water at concentrations of 0, 0.5%, 1.0%, 1.5%, 2.0%, or 3.0% (wt/wt) for a period of 14 days. Rats were 7 weeks of age at the start of the dosed water treatment, all rats survived until the end of the study, and no nonneoplastic lesions were observed by histopathology.

Aloe vera gel. The bulk Aloe vera gel extract test material had a malic acid content of 116 – 212 mg/g (mean 212 ± 1.3 mg/g) and an aloin A content of 1.1 – 1.4 mg/g (mean 1.1 ± 0.1 mg/g). Drinking water solutions of 0.5, 1.0, 1.5, 2.0, and 3.0% Aloe vera gel had malic acid contents of 1060, 2120, 3180, 4240, and 6360 μg/g water, respectively, and aloin A contents of 5.6, 11.1, 16.7, 22.2, and 33.3 μg/g water, respectively. Mean body weights, body weight gains, water consumption, feed consumption, organ weights, and gastrointestinal transit times were similar to controls. Dose-related increases in urine glucose levels were observed in female rats. Serum levels of triglycerides, cholesterol, and albumin showed dose-related decreasing trends, and triglyceride levels were significantly lower than controls at Aloe vera gel concentrations of 1.5%, 2.0% in female rats and of 3.0% in male and female rats.

Aloe vera decolorized whole leaf. The bulk Aloe vera decolorized whole leaf extract test material had a malic content of 215 – 258 mg/g (mean 243 ± 10 mg/g) and the aloin A content was 0. 6 – 0.2 mg/g (mean 0.15 ± 0.01). Drinking water solutions of 0.5, 1.0, 1.5, 2.0, and 3.0% Aloe vera decolorized whole leaf extract had malic acid contents of 1240, 2480, 3720, 4960, and 7440 μg/g water, respectively, and aloin A contents of 0.8, 1.5, 2.2, 3.0, and 4.5 μg/g water, respectively. Mean body weights, water consumption, feed consumption, and organ weights, urine chemistry, and gastrointestinal transit times were similar to controls. Hematology and clinical chemistry values were similar to controls, with the exception of significantly lower blood urea nitrogen levels in female rats exposed 1.5%, 2.0%, and 3.0% levels of Aloe vera decolorized whole leaf extract.

Aloe vera nondecolorized whole leaf. The bulk Aloe vera nondecolorized whole leaf extract test material had a malic acid content of 188 – 197 mg/g (mean 194 ± 4 mg/g) and an aloin A content of 14.1 – 15.9 mg/g (mean 14.1 ± 0.2 mg/g). Drinking water solutions of 0.5, 1.0, 1.5, 2.0, and 3.0% Aloe vera nondecolorized whole leaf extract had malic acid contents of 970, 1940, 2910, 3880, and 5820 μg/g water, respectively, and aloin A contents of 70, 141, 212, 282, and 422 μg/g water, respectively. The final mean body weights and body weight gains of rats in the 3.0% Aloe vera whole leaf groups were significantly less than those of controls; final mean body weights relative to controls were 79% in males and 81% in females. Water consumption by 3% Aloe vera whole leaf female rats and feed consumption by males exposed to 3.0% Aloe vera whole leaf extract were significantly less than those of controls. The liver, heart, spleen, thymus, and kidney weights of males and females exposed to 3.0% Aloe vera whole leaf extract were less than those of controls. Gastrointestinal tract transit times were shorter and urine volumes in male and female rats exposed to 3.0% Aloe vera whole leaf extract were lower than those of controls. Leukocyte and erythrocyte counts and hematocrit percentages were significantly elevated in male and female rats exposed to 3.0% Aloe vera whole leaf extract and creatinine and creatinine kinase values of 3.0% male rats were lower than control values.

14-DAY STUDY IN MICE

Groups of four male and four female B6C3F$_1$ mice were administered Aloe vera gel, Aloe vera nondecolorized whole leaf, or Aloe vera decolorized whole leaf extracts in drinking water at concentrations of 0, 0.5%, 1.0%, 1.5%,

2.0%, or 3.0% (wt/wt) for a period of 14 days. Mice were 7 weeks of age at the start of the dosed water treatment, all mice survived until the end of the study, and no nonneoplastic lesions were observed by histopathology.

Aloe vera gel. The bulk Aloe vera gel extract test material had a malic acid content of 116 – 212 mg/g (mean 212 ± 1.3 mg/g) and an aloin A content of 1.1 – 1.4 mg/g (mean 1.1 ± 0.1 mg/g). Drinking water solutions of 0.5, 1.0, 1.5, 2.0, and 3.0% Aloe vera gel extract had malic acid contents of 1060, 2120, 3180, 4240, and 6360 μg/g water, respectively, and aloin A contents of 5.6, 11.1, 16.7, 22.2, and 33.3 μg/g water, respectively. Mean body weights, body weight gains, water consumption, feed consumption, organ weights, hematology, clinical chemistry, urine chemistry, and gastrointestinal transit times of male and female mice were similar to controls.

Aloe vera decolorized whole leaf. The bulk Aloe vera decolorized whole leaf extract test material had a malic content of 215 – 258 mg/g (mean 243 ± 10 mg/g) and the aloin A content was 0. 6 – 0.2 mg/g (mean 0.15 ± 0.01). Drinking water solutions of 0.5, 1.0, 1.5, 2.0, and 3.0% Aloe vera decolorized whole leaf extract had malic acid contents of 1240, 2480, 3720, 4960, and 7440 μg/g water, respectively, and aloin A contents of 0.8, 1.5, 2.2, 3.0, and 4.5 μg/g water, respectively. Mean body weights, body weight gains, water consumption, feed consumption, organ weights, hematology, clinical chemistry, and urine chemistry, and gastrointestinal transit values of male and female mice were similar to controls.

Aloe vera nondecolorized whole leaf. The bulk Aloe vera nondecolorized whole leaf extract test material had a malic acid content of 188 – 197 mg/g (mean 194 ± 4 mg/g) and an aloin A content of 14.1 – 15.9 mg/g (mean 14.1 ± 0.2 mg/g). Drinking water solutions of 0.5, 1.0, 1.5, 2.0, and 3.0% Aloe vera nondecolorized whole leaf extract had malic acid contents of 970, 1940, 2910, 3880, and 5820 μg/g water, respectively, and aloin A contents of 70, 141, 212, 282, and 422 μg/g water, respectively. Mean body weights, body weight gains, feed consumption, organ weights, hematology, clinical chemistry, urine chemistry, and gastrointestinal transit values of male and female mice were similar to those of controls. Water consumption of male and female mice showed significant dose-related increasing trends, and water consumption was significantly high than controls for female mice that received the 2.0% Aloe vera nondecolorized whole leaf.

13-WEEK STUDY IN RATS

Groups of 12 male and 12 female F344/N rats were administered Aloe vera nondecolorized whole leaf extract in drinking water at concentrations of 0, 1%, 2%, or 3% (wt/wt) for a period of 13 weeks. The bulk Aloe vera nondecolorized whole leaf extract test material had a malic acid content of 170.7 – 192.9 mg/g (mean 183 ± 7) and an aloin A content of 12.6 – 13.2 mg/g (mean 12.9 ± 0.3). Drinking water solutions of 1.0, 2.0, and 3.0% Aloe vera nondecolorized whole leaf extract had malic acid contents of 1830, 3660, and 5490 μg/g water, respectively, and aloin A contents of 129, 258, and 387μg/g water, respectively.

Two male and four female rats in the 2.0% and five male and eight female rats in the 3.0% Aloe vera nondecolorized whole leaf extract groups died or were removed due to morbidity before the end of the study. Final mean body weights and body weight gains of exposed male and female rats were significantly less than those of controls; final mean body weights of 3.0% Aloe vera whole leaf exposed groups were 71.8 % of control male levels and 77.4%% of control female levels. Water consumption by exposed male rats was higher than those of controls. Mean water consumption of 3% males was approximately 2-fold higher than that of controls. Average daily doses of Aloe vera whole leaf extract over the course of the study were 1.1, 2.7, and 3.8 g/kg body wt for male rats and 1.3, 4.0, and 3.2 g/kg body wt for female rats. Volumes of 24 h urine collections of male and female rats exposed to 2% Aloe vera nondecolorized whole leaf extract were significantly lower than those of controls, and urine creatinine and glucose levels depressed. Decreased gastrointestinal transit times were observed in Aloe vera nondecolorized whole leaf exposed male and female rats; 2% male and female transit times were 4.3 and 6.2 h, respectively, compared to 11.5 and 11.0 h for control male and female rats, respectively. Hematology values for leukocyte counts, neutrophil percent, and erythrocyte counts were significantly elevated in male and female rats when compared to controls, and cholesterol and albumin levels were lower than controls. Absolute organ weights for brain, liver, heart, spleen, and thymus of rats exposed to 2% and 3% Aloe vera nondecolorized whole leaf extract were significantly less than those of controls. The incidences and severities of goblet cell hyperplasia in the large intestine of male and female rats exposed to Aloe vera whole leaf extract were increased compared to controls. There were no incidences of goblet cell hyperplasia of the large intestine in control male rats and an incidence of one (1/12, 8.3%) in the cecum of female rats; incidences were 100% for male and female rats treated with the 2% and 3% Aloe nondecolorized whole leaf extract.

13-WEEK STUDY IN MICE

Groups of 12 male and 12 female B6C3F$_1$ mice were administered Aloe vera nondecolorized whole leaf extract in drinking water at concentrations of 0, 1%, 2%, or 3% (wt/wt) for a period of 13 weeks. The bulk Aloe vera nondecolorized whole leaf extract test material had a malic acid content of 170.7 – 192.9 mg/g (mean 183 ± 7) and an aloin A content of 12.6 – 13.2 mg/g (mean 12.9 ± 0.3). Drinking water solutions of 1.0, 2.0, and 3.0% Aloe vera nondecolorized whole leaf extract had malic acid contents of 1830, 3660, and 5490 μg/g water, respectively, and aloin A contents of 129, 258, and 387μg/g water, respectively.

All mice survived until the end of the study. Mean body weights of exposed groups were similar to those of controls. Water consumption by female mice exposed to Aloe vera nondecolorized whole leaf extract was significantly higher than that of controls. Average daily doses of Aloe vera nondecolorized whole leaf extract over the course of the study were 3.7, 7.3, and 9.1 g/kg body wt for male mice and 3.7, 7.6, and 9.5 g/kg body wt for female rats. Gastrointestinal transit times of exposed 3% mice were similar to those of controls. Significant increases in 24 h urine levels of creatinine and micro protein were observed compared to those of controls. The incidences and severities of goblet cell hyperplasia in the cecum and large intestine of male and female mice exposed to Aloe vera whole leaf extract were increased compared to controls.

2 YEAR STUDY IN RATS

Groups of 48 male and 48 female F344/N rats were administered Aloe vera nondecolorized whole leaf extract at concentrations of 0, 0.5%, 1.0%, or 1.5% (wt/wt) in drinking water. The bulk Aloe vera nondecolorized whole leaf extract test material had a malic acid content of 181 – 211 mg/g (mean 195 ± 4) and an aloin A content of 5.5 – 7.6 mg/g (mean 6.3 ± 0.2). Drinking water solutions of 0.5, 1.0, and 1.5% Aloe vera nondecolorized whole leaf extract had average malic acid contents of 975, 1945, and 2920 μg/g water, respectively, and average aloin A contents of approximately 32.3, 65.6, and 98.3 μg/g water, respectively.

Survival of all exposed groups of male rats was generally similar to controls. Reduced survival was observed for the 1.5% female dose group. Mean body weight gains of 1.5% groups of exposed female rats were less than the control

group. Significantly lower feed consumption was observed for the 1.5% Aloe vera nondecolorized whole leaf extract treatment groups of male and female rats when compared to those of controls; however, daily feed consumptions over the 104 week study were approximately 90% of control levels. Water consumptions by male rats exposed to 1.0% and by male and female rats exposed to 1.5% Aloe vera nondecolorized whole leaf extract were significantly higher than those of controls. Mean daily water consumptions of 1.0% and 1.5% male rats in the 104 week study were 27 and 31 g, respectively for males; mean daily water consumption of male control rats was 22 g.

Treatment-related neoplasms and nonneoplastic lesions that occurred in the rat were primarily in the large intestine. Incidences of carcinomas of the ascending colon in 1.5% Aloe vera nondecolorized whole leaf extract groups of male and female rats were higher than that of controls. The incidences of adenomas of the proximal colon in 1.0% and 1.5% groups of male and 1.5% group of female rats were higher than that in controls. Incidences of adenomas of the transverse colon in 1.0% male rats were higher than the control group. The incidences of all adenomas, all carcinomas, or the combined incidences of adenomas and carcinomas of the proximal, cecum, ascending, and transverse colon were significantly higher than controls in both male and female rats in the 1.0% and 1.5% dosed groups. Incidences of adenomas and/or carcinomas combined were 21% and 39% in female rat 1.0% and 1.5% Aloe vera nondecolorized whole leaf extract groups, respectively, and 67% and 74% in male rat 1.0% and 1.5% Aloe vera whole leaf extract groups, respectively. Neoplasms of the large intestine were not observed in control animals.

In male and female rats exposed to Aloe vera whole leaf extract, dose-related incidences of mucosal hyperplasia of the proximal colon, cecum, ascending, transverse, and descending colon sites were significantly higher than that of controls. The incidences of cystic mesenteric lymph node degeneration and cecal dilatation were higher in the 1.0% and 1.5% Aloe vera nondecolorized whole leaf extract groups of male and female rats than that in controls.

2-YEAR STUDY IN MICE

Groups of 48 male and 48 female B6C3F_1 mice were administered Aloe vera nondecolorized whole leaf extract at concentrations of 0, 1.0%, 2.0%, or 3.0% (wt/wt) in drinking water for 2 years. The bulk Aloe vera nondecolorized whole leaf extract test material had a malic acid content of 181 – 211 mg/g (mean 195 ± 4) and an aloin A content of 5.5 – 7.6 mg/g (mean 6.3 ± 0.2). Drinking water solutions of 1.0, 2.0, and 3.0% Aloe vera nondecolorized whole

leaf extract had average malic acid contents of approximately 1945, 3640, and 5835 μg/g water, respectively, and average aloin A contents of approximately 65.6, 131.3, and 196.8 μg Aloe vera nondecolorized whole leaf/g water, respectively.

Survival of all exposed groups was similar to that of controls. Mean body weight gains of male mice were less than those of controls, but ranged from 93 – 95% of control body weights. Feed consumption by 2.0% and 3.0 Aloe vera nondecolorized whole leaf extract mouse groups was higher than that of controls. Polydipsia was pronounced in both sexes administered the Aloe vera whole leaf extract, and water consumption by male and female mice exposed to Aloe vera nondecolorized whole leaf extract ranged from approximately 150% – 260% of control levels and equated to average daily doses of 2.5 – 11 g of Aloe vera nondecolorized whole leaf extract/kg body weight.

There were no significant increased incidences of neoplasms in mice in response to the Aloe vera nondecolorized whole leaf extract treatment in the drinking water. Treatment related increasing trends in the incidences of goblet cell hyperplasia were observed in the colons of male and female mice. The significance of this finding is uncertain.

CONCLUSIONS

Under the conditions of these 2-year drinking water studies, there was *clear evidence of carcinogenic activity* of a nondecolorized whole leaf extract of Aloe vera in male and female F344/N rats based upon increased incidences of adenomas and carcinomas of the large intestine. There was *no evidence of carcinogenic activity* in male or female B6C3F_1 mice exposed to 1.0%, 2.0%, or 3.0 % (wt/wt) Aloe vera whole leaf extract in drinking water.

Exposure to a nondecolorized whole leaf extract of Aloe vera resulted in increased incidences of nonneoplastic lesions of the large intestine in male and female rats and mice, the small intestine of male and female rats, the stomach in male and female rats and female mice, the mesenteric lymph nodes in male and female rats and male mice, and the nose in male mice.

Summary of the 2-Year Carcinogenesis Study of Aloe vera

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses in drinking water	0, 0.5, 1.0, or 1.5% (wt/wt)	0, 0.5, 1.0, or 1.5% (wt/wt)	0, 1.0, 2.0, or 3.0% (wt/wt)	0, 1.0, 2.0, or 3.0% (wt/wt)
Body weights	All dose groups remained within 10% of controls	1.0% dose group 10% less than the control group after week 100 and 14% below those of controls by week 104; and 1.5% dose group 14% less than control group and 20% below those of controls by week 104	All dose groups remained within 10% of controls.	All dose groups remained within 10% of controls.
Survival rates	15/38, 17/48, 19/48, 15/48	30/48, 31/48, 24/48, 20/48	31/48, 28/47, 21/48, 28/48	35/47, 30/48, 36/48, 34/48
Nonneoplastic effects	Mesenteric lymph node: hyperplasia (0/47, 0/48, 1/48, 4/48); cystic degeneration (8/47, 11/48, 42/48, 41/48); Glandular stomach: mucosa hyperplasia (1/48, 12/47, 7/48, 11/48) Small intestine: jejunum mucosa hyperplasia (0/45, 1/44, 2/46, 3/46) Large intestine: proximal colon mucosa hyperplasia (0/44, 29/44, 36/46, 32/41); cecum mucosa hyperplasia (0/46, 13/45, 24/48, 25/48); ascending colon mucosa hyperplasia (0/47, 30/47, 38/48, 32/46); transverse colon mucosa hyperplasia (0/47, 30/47, 42/47, 34/47); descending colon mucosa hyperplasia (0/47, 17/46, 31/46, 30/47); colon mucosa hyperplasia (0/0, 1/1, 1/3, 4/5); rectum mucosa hyperplasia (0/47, 1/47, 1/48, 4/48); cecum dilatation (1/46, 0/45, 8/48, 17/48);	Mesenteric lymph node: hyperplasia (0/46, 2/47, 2/48, 3/47); cystic degeneration (0/46, 16/47, 40/48, 43/47); Glandular stomach: mucosa hyperplasia (0/48, 1/48, 3/48, 3/48); forestomach inflammation (0/48, 0/48, 4/48, 3/48); forestomach hyperplasia (1/48, 7/48, 10/48, 9/48) Small intestine: ileum mucosa hyperplasia (0/47, 2/48, 2/43, 6/44) Large intestine: proximal colon mucosa hyperplasia (0/43, 30/45, 33/42, 32/39); cecum mucosa hyperplasia (0/47, 4/48, 17/47, 27/48); ascending colon mucosa hyperplasia (0/47, 40/48, 35/46, 39/46); transverse colon mucosa hyperplasia (0/47, 40/48, 33/46, 42/46); descending colon mucosa hyperplasia (0/47, 17/48, 18/46, 27/47); rectum mucosa hyperplasia (0/48, 0/48, 0/47, 5/47); cecum dilatation (0/47, 0/48, 9/47, 25/48); proximal colon inflammation (0/43, 2/45, 11/42, 8/39);	Mesenteric lymph node: cellular infiltration (0/48, 1/45, 4/45, 6/43) Large intestine: ascending colon, goblet cell hyperplasia (2/47, 16/44, 20/45, 19/42); transverse colon goblet cell hyperplasia (4/47, 14/44, 21/45, 22/43); descending colon, goblet cell hyperplasia (0/47, 7/44, 12/45, 17/43); Nose: hyaline droplet (6/48, 31/47, 39/47, 13/47)	Glandular stomach: epithelial hyperplasia (0/43, 1/44, 3/45, 4/42) Large intestine: ascending colon, goblet cell hyperplasia (1/43, 15/43, 20/44, 25/43); transverse colon, goblet cell hyperplasia (2/42, 18/42, 23/44, 26/43); descending colon, goblet cell hyperplasia (0/43, 4/43, 7/44, 17/43);

Summary of the 2-Year Carcinogenesis Study of Aloe vera

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic effects	Large intestine: proximal colon adenoma (0/44, 0/44, 7/46, 10/41); proximal colon carcinoma (0/44, 0/44, 4/46, 4/41); cecum adenoma (0/46, 0/45, 8/48, 8/48); cecum carcinoma (0/46, 0/45, 1/48, 2/48); ascending colon adenoma (0/47, 0/47, 19/48, 8/46); ascending colon carcinoma (0/47, 0/47, 4/48, 8/46); transverse colon adenoma (0/47, 0/47, 6/47, 3/47); transverse colon carcinoma (0/47, 0/47, 1/47, 1/47); adenoma (0/47, 0/48, 26/48, 23/48); carcinoma (0/47, 0/48, 10/48, 14/48); Adenoma and carcinoma combined (0/47, 0/48, 28/48, 31/48)	Large intestine: proximal colon adenoma (0/43, 0/45, 4/42, 5/39); proximal colon carcinoma (0/43, 0/45, 2/42, 4/39); cecum adenoma (0/47, 0/48, 1/47, 6/48); ascending colon adenoma (0/47, 0/48, 1/46, 5/46); adenoma (0/48, 0/48, 6/48, 13/48); carcinoma (0/48, 0/48, 3/48, 4/48); Adenoma and carcinoma combined (0/48, 0/48, 8/48, 15/48)	None	None
Equivocal findings	None	None	None	None
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	No evidence	No evidence

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major flaws **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised on March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS PEER REVIEW PANEL

The members of the Technical ReportsPeer Review Panel who evaluated the draft NTP Technical Report on Aloe vera on April 5, 2011, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

SUMMARY OF TECHNICAL REPORTS PEER REVIEW PANEL COMMENTS

NOTE: A summary of the Technical Reports Peer Review Panel's remarks will appear in a future draft of this report.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

Aloe, a genus within the Liliaceae family, is composed of approximately 420 species of plants. *Aloe barbadensis* Miller, Aloe vera, is one species of Aloe. Other common names of *Aloe barbadensis* Miller include Barbados aloe, Mediterranean aloe, True aloe, and Curacao aloe. Aloes are perennial succulents or xerophytes; they are adaptable to habitats with low or erratic water availability, are characterized by the capacity to store large volumes of water in their tissue, and are able to utilize crassulacean acid metabolism, an adaptation to the photosynthetic pathway that involves the formation of malic acid (Alves *et al.*, 2004; 2005). Aloe plants, such as Aloe vera, have in common green fleshy leaves covered by a thick cuticle or rind and an inner clear pulp. The vascular bundles, located within the leaf pulp, transport water and minerals from the roots to the leaves; transport synthesized materials to the roots; and transport the latex along the margins of the leaf for storage (Ni *et al.*, 2004) (Figure 1). The number of vascular bundles varies depending on the size of the leaves and the age of the plant (Ni *et al.*, 2004).

The main feature of the Aloe vera plant is its high water content, ranging from 99%-99.5% (Atherton, 1998). The remaining 0.5%-1.0% solid material is reported to contain over 75 different potentially active compounds, including vitamins, minerals, enzymes, simple and complex polysaccharides, phenolic compounds, and organic acids. In compositional studies on the structural components of the Aloe vera plant leaf portions, the rind was found to compose 20%-30% and the pulp 70%-80% of the whole leaf weight. On a dry weight basis, the percentages of the rind and pulp that was represented as lipids (2.7% and 4.2%) and that as proteins (6.3% and 7.3%) only accounted for a minor fraction (Femenia *et al.*, 1999). The percentages of soluble sugars (11.2% and 16.5%), primarily as glucose, and the percentages of ash (13.5% and 15.4%), in particular calcium, were relatively high in the rind and pulp, respectively. Non-starch polysaccharides and lignin represented the bulk of each leaf fraction and were found to be 62.3% and 57.6% of the dry weight of the rind and pulp, respectively.

Two commercially important products are obtained from the leaves of the Aloe vera plant: gel and latex. The physical and chemical constituents of the products derived from the Aloe vera plant differ depending on the source (e.g. part of the plant), the species of the plant, the climate, and the growing conditions (Klein and Penneys, 1988; Shelton, 1991; Briggs, 1995). A two-year study of the Aloe vera plant found fluctuations in several physical and chemical properties attributable to seasonal and grower influences (Wang and Strong, 1995). The average leaf weight was found to increase and total and soluble solids were found to decrease during the winter months. Fluctuations in mineral concentrations were attributable to horticultural conditions, such as crop rotation and fertilization methods, rather than irrigation practices. Limitation in light availability was found to affect total dry mass production and carbon allocation primarily, such as the number of leaves per plant (Paez *et al.*, 2000). The percentages of carbon distribution within plants that were grown in full sunlight were 53% in the leaves and 28% in the roots; while that of plants grown in partial shade was 70% in the leaves and only 13% in the roots. Genet and van Schooten (1992) reported that an increase in hydration of the Aloe vera plant resulted in increases in leaf thickness and gel production; in contrast, over exposure to a combination of sunlight and drought conditions resulted in low aloe gel yield.

Aloe Vera Gel Extract

The inner leaf pulp of the Aloe vera plant leaf contains large, thin-wall cells that produce Aloe vera gel, the clear, mucilaginous, and aqueous extract of the inner central area of the leaf pulp (Figure 1). Aloe vera gel serves as the water and energy storage component of the plant (Yaron, 1993; Paez *et al.*, 2000). The mechanical extrusion of the mucilaginous gel from the inner leaf pulp gives a 70% yield with a water content of 99%-99.5% (Femenia *et al.*, 1999). The gel of field-grown Aloe vera is reported to have a pH of 4.4-4.7 and a total and soluble solids content of 0.56%-0.66%; however, seasonal fluctuations and fluctuations due to water availability were noted (Yaron, 1993; Wang and Strong, 1995; Waller *et al.*, 2004). The high acidity of the Aloe vera gel may be due to the accumulation of organic acids, such as malic acid, via crassulacean acid metabolism. Chemical analysis of the gel extract indicates that, as with the rind and pulp, lipids and proteins are minor fractions of the dry weight, representing 5.1% and 8.9%, respectively; however, the amount of soluble sugars (27.8%) detected is substantially higher than that in the rind or pulp (Wang and Strong, 1995). The ash content is relatively high in all fractions of the plant, but in particular in the gel, where it accounts for 23.6% of the dry matter. Calcium is the main mineral present in the rind and pulp fractions, whereas, sodium and potassium are higher in the aloe gel. The reasons for the predominance of

these minerals in the gel is unclear; however, sodium is known to have a role in water distribution and potassium is thought to improve tissue repair (Robson *et al.*, 1982). Non-starch polysaccharides and lignin represent 35% of the dry mass of the gel (Femenia *et al.*, 1999).

Aloe vera gel polysaccharides consist of linear chains of glucose and mannose molecules, and, because there is considerably more mannose present than glucose, the molecules are referred to as polymannans. These linear chains range in size from a few to several thousand molecules. The major polysaccharide, acemannan, is composed of one or more polymers of various chain lengths with molecular weights ranging from 30-40 kDa or greater and consisting of repeating units of glucose and mannose in a 1:3 ratio (Gowda *et al.*, 1979; Mandal and Das, 1980; Yaron, 1993; Femenia *et al.*, 1999; Chow *et al.*, 2005). The polysaccharide sugar moieties of acemannan are linked by beta (β) glycosidic bonds to form linear chains with random O-acetyl groups and a low degree of galactose side chain branching. The β-1→4 glycosidic bond configuration of acemannan is an important consideration when examining the reported therapeutic effects of Aloe vera gel, since humans lack the capacity to enzymatically digest these bonds. The size and structure of the polysaccharide polymers result in the formation of a colloidal system within the leaf pulp tissue that increases the viscosity and opacity of the mostly aqueous solution (Danhof, 1998). The chemical bonds, within the carbohydrate polymers contribute to these qualities, but are susceptible to degradation by endogenous and exogenous bacteria (Gorloff, 1983; Yaron, 1993; Waller *et al.*, 2004). Chemically preserved fresh aloe gel stored at room temperature or incubated at 40° C for 48 hours exhibited degradation in its rheological properties, a decrease in the content and composition of polysaccharides, and a substantial increase in the mannose:glucose ratio, from 2.9 in the fresh gel to 13.4 in the incubated gel (Yaron, 1993). Ross *et al.* (1997) examined a number of commercial Aloe vera gel products using size exclusion chromatography and found a wide disparity in the levels acemannans; some products had levels below the detection limits. Similarly, Turner *et al.* (2004) found significant variation in commercial product content when compared with plant-derived native aloe gel.

Aloe Vera Nondecolorized Whole Leaf Extract

The Aloe vera nondecolorized whole leaf extract, commonly referred to as whole leaf Aloe vera juice or Aloe juice, is the aqueous extract of the whole Aloe vera leaf with lignified fibers removed. The Aloe vera whole leaf extract contains both the gel from the inner parenchyma leaf pulp and the latex. Aloe vera latex is a bitter, yellow plant

exudate that is stored and transported along the margins of the Aloe vera leaf via pericyclic tubules within the vascular bundles, which are located within the leaf pulp beneath and adjacent to the leaf rind (Figure 1). The restricted distribution of the bitter latex within the margins of the leaves of the Aloe vera plant suggests that it is a source of secondary metabolites: compounds that do not function directly in plant growth and development and serve as a plant defense strategy (Chauser-Volfson and Gutterman, 1996; Wink, 2003). A wide variety of secondary compounds have been isolated from the aloe latex (Reynolds, 1985). The isolated compounds are largely phenolic in nature, and many are anthraquinone *C*-glycosides, anthrones, and free anthraquinones (Park *et al.*, 1998). The levels of anthraquinone C-glycosides in aloe latex are quite variable; however, they may constitute up to 30% of the dry weight of aloe latex (Groom and Reynolds, 1986). Aloe vera latex contains four major C-glycosyl constituents: aloin A, aloin B, aloesin, and aloeresin A (Figure 2) (Saccu *et al.*, 2001). Aloin A, a C-glycosyl anthrone, also referred to as barbaloin, is the major component of aloe latex (Birch and Donovan, 1955; Hay and Haynes, 1956; Reynolds, 1985). Aloin A and its epimer, Aloin B, also referred to as isobarbaloin (Figure 2) have a 9-anthrone skeleton and a β-D-glucopyranosyl substituent (Manitto *et al.*, 1990). Aloesin, also known as aloeresin B, is a 5-methyl chromone with an 8-β-D-glucopyranosyl substituent (Haynes and Holdsworth, 1970), and aloeresin A is a 5-methyl chromone with an 8-β-D-glucopyranosyl -2-O-*trans*-*p*-coumarol substituent (Gramatica *et al.*, 1982). Several other C-glycosyl-chromones and anthrones have been isolated from Aloe vera, including aloe-emodin, the anthraquinone of barbaloin and isobarbaloin (Zonta *et al.*, 1995; Okamura *et al.*, 1996; Okamura *et al.*, 1997; Saleem *et al.*, 1997; Park *et al.*, 1998).

The occurrence in Aloe vera latex of endogenous free anthraquinones and anthrones results from oxidative processes rather than from metabolic synthesis (Franz and Grun, 1983; Hattori *et al.*, 1988; Saleem *et al.*, 1997). In addition, the latex from Aloe vera contains a number of aromatic compounds, such as aldehydes and ketones (Saccu *et al.*, 2001). On a dry weight basis, the aloe latex is reported to also contain an acid insoluble resin (16-63%), significant ash content (24.5%), and a small quantity of essential oil that is responsible for the odor of the latex (Mapp and McCarthy, 1970). The sugar moiety in aloins is D-glucose, and studies indicate that carbon atom 1 of the D-glucose moiety is linked directly to carbon atom 10 of the anthracene ring in a β-configuration (Figure 2). The carbon–carbon bond is quite resistant to acid and alkaline conditions, and cleavage by oxidation, rather than hydrolysis, is achieved only under the drastic conditions of acid in combination with an oxidant (Hay and Haynes, 1956). The β-

(1→10) C–C bond is also resistant to β–glycosidase of plants and most plant bacteria (Vyth and Kamp, 1979; Joshi, 1998); however, the intestinal micro flora of humans and animals have been shown to cleave the β-C-glucosyl bond, although considerable variation in response among animal species occurs (Mapp and McCarthy, 1970; Hattori *et al.*, 1988; Che *et al.*, 1991). Cleavage of the β-C-glucosyl bond results in the formation of aloe-emodin, the cathartic principle of the latex, and other free anthraquinones and anthrones (Figure 2).

Aloe Vera Decolorized Whole Leaf Extract

Activated carbon adsorption of the Aloe vera nondecolorized whole leaf extract to remove the anthraquinone components of aloe latex results in a product termed decolorized whole leaf extract that has quite different properties. Aloe vera decolorized whole leaf extract is also referred to as whole leaf Aloe vera gel. According to an Aloe vera trade group website (http://www.iasc.org accessed on February 10, 2011), the standard for aloin content in Aloe vera decolorized whole leaf products for oral consumption is less than 10 ppm (parts per million). Although Aloe vera gel and the decolorized whole leaf extract are similar in that each contain little or no aloe latex anthraquinones, carbon adsorption changes the physical and chemical properties of the Aloe vera whole leaf extract. Aloe vera decolorized whole leaf differs from Aloe vera gel in that it exhibits a degradation in rheological properties and a loss of approximately 19% - 23% of the complex polysaccharide content (Pelley *et al.*, 1998).

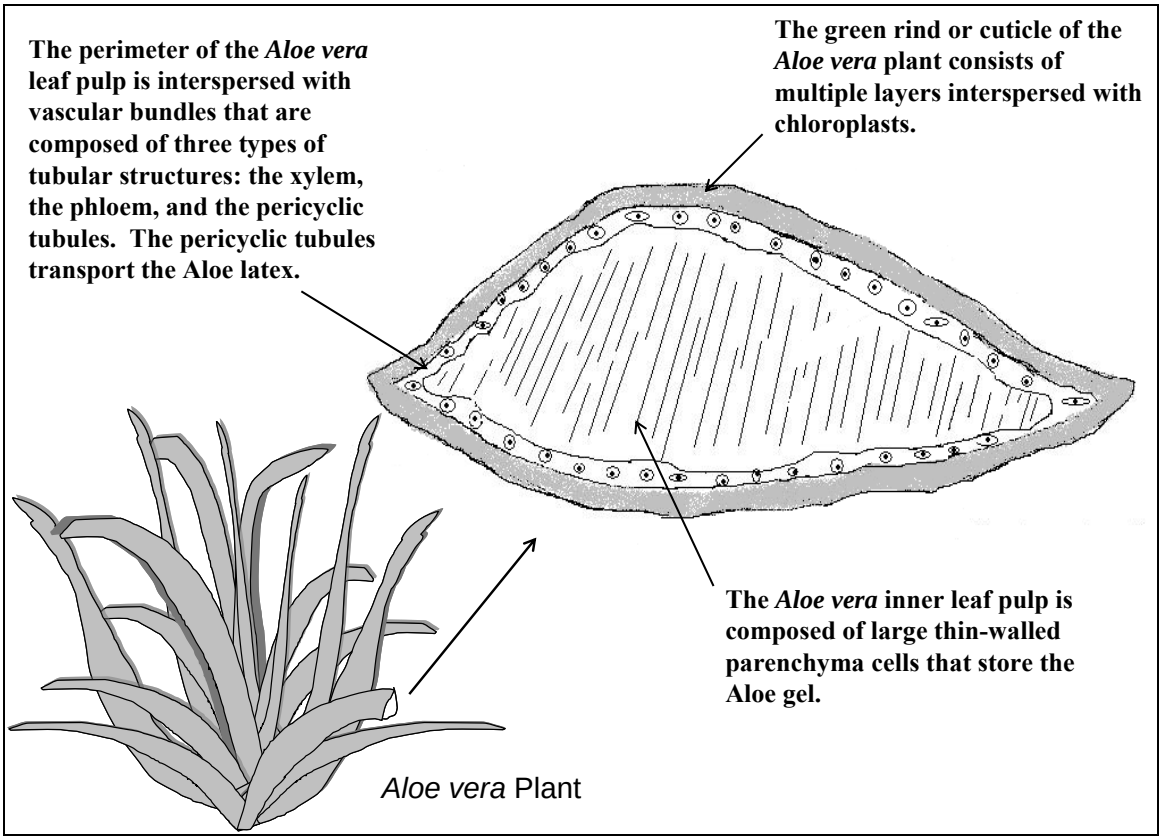


FIGURE 1
Schematic representation of the Aloe vera plant and a cross-section through an Aloe vera leaf.

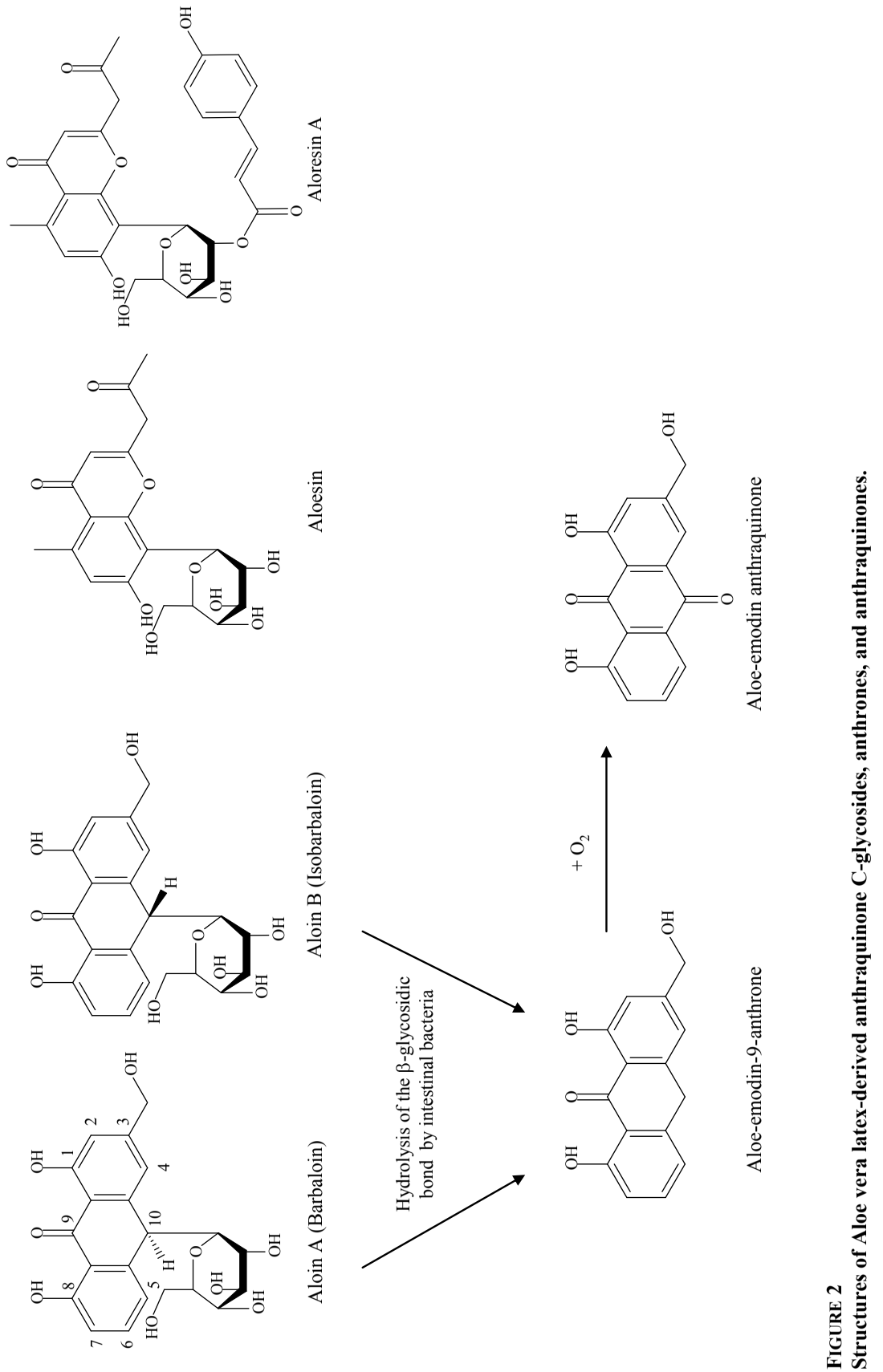


FIGURE 2
Structures of Aloe vera latex-derived anthraquinone C-glycosides, anthrones, and anthraquinones.

PRODUCTION, USE, AND HUMAN EXPOSURE

Aloe vera is one of approximately 420 species of Aloe that are now indigenous to dry sub-tropical and tropical climates, including the southern United States (Grindlay and Reynolds, 1986; Viljoen and Van Wyk, 2000). Among the Aloe species, Aloe vera is the most widely used both commercially and for its therapeutic properties (Eshun and He, 2004; Ni *et al.*, 2004). Commercial cultivation of Aloe vera in the United States began in the 1920s in Florida (Grindlay and Reynolds, 1986). Aloe vera has become an important plant crop in Arizona and in the Rio Grande valley of southern Texas. Other Aloe species grown for commercial use include *Aloe andongensis*, *Aloe arborescens*, *Aloe perryi*, and *Aloe ferox*. These species differ from *Aloe barbadensis* Miller, Aloe vera, in their composition, which can be confusing to the consumer since the literature often includes these species as synonyms for aloes and Aloe vera. The U.S. Food and Drug Administration (FDA) permits the use of *Aloe barbadensis, Aloe ferox*, and *Aloe perryi* as food additives for human consumption as natural flavor enhancers (CIR, 2007). A published tabulation of acceptable levels of natural flavorings by the Flavor and Extract Manufacturers' Association indicates that for Aloe vera extract an acceptable level is 5 – 2000 ppm. No distinction is given for the part of the plant or type of plant extract used to produce the extract used as a flavoring additive (Duke and Beckstrom-Sternberg, 1994).

Aloe vera grows best in dry chalky soil or in a sandy loam (Grindlay and Reynolds, 1986). While the plant needs warm semi-tropical conditions, overexposure to sun results in stunted plants with low gel yield. Therefore, Aloe vera is commonly interplanted with other crops, such as fruit trees. Considerable variation in the quality of Aloe vera plant products exists due to differences in growing, harvesting, processing, and storage techniques. Harvesting of Aloe vera plant leaves is generally performed by hand, with the leaves cut from the base of the plant (Grindlay and Reynolds, 1986). Individual leaves are wrapped, crated, and transported to processing plants. Ideally, Aloe vera leaves are processed within a few hours of harvesting, as temperature, light, air, and humidity can affect the stability of the Aloe vera plant components (Paez *et al.*, 2000). At the processing step, the leaves are cleaned with water and a mild chlorine solution.

Aloe vera gel from the fillet of the inner leaf pulp is obtained either by manual removal of the outer layers of the leaf with a knife or by machine. Either method can be flawed and has the potential to contaminate the aloe gel with aloe latex (Grindlay and Reynolds, 1986). This process yields crude Aloe vera gel. High quality Aloe vera gel appears opaque, slightly off-white in color, and is viscous (Vogler and Ernst, 1999).

Aloe vera whole leaf extract is obtained by grinding the whole fresh leaves, without removal of the rind. Extraneous material and lignified fibers are then removed by homogenizing and filtering the crude gel or whole leaf extracts (Yaron, 1993). Since various amounts of aloe latex and rind may be present in the whole leaf extracts, the extracts may appear yellow to yellow-green in color.

Activated carbon adsorption to produce Aloe vera decolorized whole leaf extract is the first processing step where an extract is intentionally subjected to chemical alteration. Aloe vera decolorized whole leaf has lower rheological values than aloe gel and has a lower content of complex carbohydrates than either aloe gel or whole leaf extracts (Pelley *et al.*, 1998).

The processed extracts are difficult to keep stable, a problem that may cause differences in product potency; therefore, the gel or whole leaf extracts can undergo a "stabilization" process before being bottled. This process involves pasteurization, ultraviolet stabilization, chemical oxidation with hydrogen peroxide, adulteration with chemical preservatives and additives, or concentration, and/or drying (Gorloff, 1983; Grindlay and Reynolds, 1986; Yaron, 1993; Simal *et al.*, 2000).

The Aloe vera plant has been used in folk medicine for over 2000 years, and the Aloe vera plant remains an important component in traditional medicine of many contemporary cultures, such as China, India, the West Indies, and Japan (Grindlay and Reynolds, 1986). Both classes of leaf products, Aloe vera gel and Aloe vera latex, are reported to possess a wide range of pharmaceutical activities. In recent times, the oral consumption of Aloe vera has been promoted as a prophylaxis and treatment to alleviate a variety of unrelated systemic conditions (Marshall, 1990). Promoters offer a number of Aloe vera whole leaf formulations that are widely available for consumption at various concentrations in liquid, powder, and tablet form. Reports credit Aloe vera with anti-tumor (Imanishi *et al.*,

1981; Imanishi and Suzuki, 1984; Imanishi and Suzuki, 1986; Imanishi *et al.*, 1986; Kim *et al.*, 1999; Zhao *et al.*, 1999; Keum *et al.*, 2000), anti-arthritic (Spoerke and Ekins, 1980; Hanley *et al.*, 1982; Davis *et al.*, 1986), anti-rheumatoid (Davis *et al.*, 1986; Davis *et al.*, 1992), (Dykman *et al.*, 1998), anti-cancer (Kim *et al.*, 1999; Pecere *et al.*, 2000), and anti-diabetic (Ghannam *et al.*, 1986; Davis *et al.*, 1988; Ajabnoor, 1990; Roman-Ramos *et al.*, 1991; Bunyapraphatsara *et al.*, 1996; Yongchaiyudha *et al.*, 1996) properties. In addition, Aloe vera is promoted for constipation and gastrointestinal disorders (Saito *et al.*, 1989; Teradaira *et al.*, 1993; Atherton, 1998) and for immune system deficiencies (Davis *et al.*, 1987b; Davis *et al.*, 1994; Hutter *et al.*, 1996). The scientific literature yields little to substantiate claims of usefulness for systemic conditions by the ingestion of Aloe vera (Hecht, 1981; Klein and Penneys, 1988).

In its dried form, Aloe vera latex is a laxative regulated as a drug by the FDA and is also used as a bitter flavoring additive by the food industry. Aloe vera gel is primarily used a topical agent for skin wounds and irritations but is also taken internally for the treatment of gastric ulcers and diabetes. Aloe vera whole leaf extract, which combines both the gel and latex, and Aloe vera decolorized whole leaf extract, which has most of the latex components removed, are popular as dietary supplements for various systemic ailments and are promoted as potential anti-cancer, anti-AIDS, and anti-diabetic agents. The anthraquinone components of these products appear to vary significantly in their content of aloe-emodin and aloin A, the major anthraquinone constituent of Aloe vera latex. ElSohly and Gul (2007) evaluated 53 liquid and 30 semisolid and solid Aloe-based commercial products. The liquid samples all contained ≤ 10 ppm of either aloe-emodin or aloin A, with many having no detectable levels of either of the two analytes. Unlike liquid products, many solid and semisolid products (11 out of 30) contained ≥ 10 ppm of one or both of the analytes, aloe-emodin and aloin A.

BIOLOGICAL PROPERTIES OF ALOE VERA GEL

Metabolism

Yagi *et al*. (1999) examined the metabolism of fluoresceinyl isothiocyanate (FITC)-labeled acemannan (500 kDa molecular weight) when administered to mice orally or by intravenous injection at a dose of 120 mg/kg. Tissue distribution analyses 2 h after intravenous administration of FITC-acemannan at a dose of 120 mg/kg indicated that the kidney was the major site of accumulation in mice and that the acemannan was metabolized into lower-

molecular-weight molecules (70 – 10 kDa) that were excreted primarily in the urine 24 h after intravenous injection in mice, with minimal amounts excreted in the feces over the 48 h period. Oral administration of the FITC-acemannan at 120 mg/kg resulted in low-molecular-weight substances (less than 9 kDa) appearing primarily in the feces in the first 24 h compared with that excreted in the urine 48 h after administration (Yagi *et al.*, 1999). An intestinal bacterial mixture from human feces was shown to metabolize acemannan (≥ 400 kDa molecular weight) to smaller components (30 and 10 kDa) in a 1% yield. Structural studies of the catabolites by 1H-NMR and IR spectroscopy and HPLC analyses indicated the presence of sugar and peptide moieties. Since humans lack the capacity to enzymatically digest the β-1→4 glycosidic bond configuration, these smaller components are likely segments of acemannan that lack the β-configuration or possibly mucin arising from the feces (Yagi *et al.*, 1999).

Cell Proliferation

There are several reports about the stimulatory effect of *Aloe* components on cell proliferation (Danhof and McAnally, 1983; Davis *et al.*, 1987a); however, the identity of the substances responsible for influencing cell proliferation is currently not known. Since no single definitive active ingredient has been identified, some suggest that there may be synergism between the polysaccharides and other components in the aloe gel; others continue to isolate and examine the various polysaccharides, proteins, and numerous other components in the Aloe vera plant products for pharmacological and physiological activities.

There are numerous reports about stimulatory and inhibitory effects of Aloe vera lectin-like substances on cell proliferation. Lectins are glycoproteins of non-immune origin that are known for their ability to agglutinate (clump) erythrocytes *in vitro*. Reduced growth, diarrhea, and interference with nutrient absorption are caused by this class of toxicants. Different lectins have different levels of toxicity, though not all lectins are toxic. Lectins may bind with free sugar or with free or bound sugar residues of polysaccharides, glycoproteins, or glycolipids in cell membranes. When given orally to experimental animals, lectins interact with the mucosa of the gastrointestinal tract causing acute gastrointestinal symptoms, failure to thrive, and even death. Lectins can alter host resistance to infection or, more importantly, to tumors. Following the initial discovery of highly toxic ricin from castor bean, lectins have been detected in a number of edible plants. The toxic effects of lectins are dependent on source, species, dose, and route of administration (Hayes, 1999).

The occurrence of lectin-like substances in Aloe vera was first described by Winters *et al.* (Winters *et al.*, 1981), who reported that fractions prepared by differential centrifugation from fresh leaf and commercial aloe gel extracts contained high levels of lectin-like substances. The fresh leaf fractions were found to promote the attachment and growth of normal human cells, but not tumor cells; while, the commercial aloe gel fractions were found equally cytotoxic to normal human and tumor cells. Substances in fractions of Aloe vera whole leaf and gel extracts were also found to induce proliferation in fibroblast and neuron-like cells (Bouthet *et al.*, 1995). Although the term lectin or glycoprotein was not mentioned, proteins in the Aloe vera extracts were measured and treatments assigned based on protein concentrations. The aloe gel was found more potent in stimulating the growth of cells, when cells were treated prior to attachment then in the treatment of adherent cell cultures. Since the adherence of cells to a matrix is an essential factor for growth, the results suggest that aloe gel may improve cell attachment. Subsequently, human fibroblast cells treated with fresh Aloe gel were shown to increase in a dose-dependent fashion, while cytotoxicity was observed in cells treated with aloe latex (Danhof and McAnally, 1983). In contrast to the effects observed with treatment of native aloe gel, a commercial gel was found to have differing effects, suggesting that substances were added during the processing that altered the lectin-like activities and resulted in the disruption of cell attachment and growth. However, when cytotoxicity assays were conducted with an *in vitro* system that mimicked human skin, the effective concentration to kill 50% of cells (EC50) could not be determined, since the aloe gel at a 100% concentration was found essentially non-toxic and actually stimulated cellular activity (Bowles, 1994).

Fractionated whole leaf and gel extracts of Aloe vera have been used to identify and characterize the Aloe vera lectin-like substances. Gel permeation was used to isolate three aloe gel fractions (1997). A glycoprotein fraction was found to promote cell growth, a colored glycoprotein fraction was found to inhibit cell growth, and a neutral polysaccharide fraction was found to neither promote nor inhibit cell growth. The colored glycoprotein fraction was found to contain phenolic components, and these components were thought to reduce the proliferative effect of the lectin-like substances in aloe gel. Using HPLC analysis, small quantities of phenolic components, including barbaloin and aloesin, were detected in virtually all samples of aloe gels tested (Okamura *et al.*, 1997). Although the

phenolic substances were detected in negligible amounts, these results suggested that the variability observed in proliferation studies on aloe gel may be explained by the presence of inhibitory phenolic substances.

Akev and Can (1999) reported on the separation and purification of two leaf pulp lectins isolated from Aloe vera, Aloctin I and Aloctin II. The lectins had a glycoprotein structure and exhibited haemagglutinating activity against rabbit erythrocytes, but failed to agglutinate human erythrocytes and only weakly agglutinated rat erythrocytes. Human foreskin keratinocytes and squamous cell carcinoma cells showed a significant proliferative response to an isolated glycoprotein fraction from aloe gel, G1G1M1D12. Using a raft culture — a synthetic mono-layer culture of keratinocytes that mimics human epidermis — Choi *et al.* (2001) demonstrated that G1G1M1D12 induced the migration of keratinocytes to restore wounded cell areas and stimulated the cells to express protein markers related to cell proliferation in a dose-dependent manner.

Angiogenesis

Angiogenesis is the growth of new capillaries from pre-existing vessels and is the summation of a multi-step process that involves the migration and proliferation of capillary endothelial cells, tissue infiltration, and lumen formation (Breier and Risau, 1996). Capillaries provide the essential interface between the blood and the tissue for regulating nutrient delivery and for the transmigration of cells (Bischoff, 1995). Therefore, while angiogenesis is essential for normal tissue growth, it also occurs in many physiological and pathological conditions, including tumor growth (Folkman and Klagsbrun, 1987).

A number of potent angiogenic compounds have been identified in Aloe vera. Moon *et al.* (1999) showed that the crude extract of aloe gel actively induced neovascularization on the chorioallantoic membrane of chick embryo. Subsequently, the aloe gel was separated into three fractions, which were tested *in vitro* and *in vivo* for angiogenic activity. Further fractionation showed that the angiogenic effect was mainly due to the plant sterol, β-sitosterol. Lee *et al.* (1998) partitioned aloe gel into three fractions and tested these fractions for *in vitro* angiogenic activity in calf pulmonary artery endothelial (CPAE) cells. One of the fractions was found to be active and induced the proliferation of CPAE cells, stimulated CPAE cells to invade the matrigel matrix, and enhanced the differentiation of CPAE cells to form capillary-like tubules. The treated CPAE cells were also shown to have enhanced mRNA expression of several angiogenic activators (Lee *et al.*, 1998).

β-Sitosterol was isolated from aloe gel and examined for its effect upon damaged blood vessels in ischaemic/reperfused brains of gerbils. The aloe gel extracted β-sitosterol was shown to enhance new vessel formation in a dose-dependent fashion (≤ 500 mg/kg) and to enhance the expression of several proteins related to angiogenesis, namely von Willebrand factors, vascular endothelial growth factor (VEGF), the VEGF receptor FLK-1, and blood vessel matrix laminin (Choi *et al.*, 2002).

Immune System

Anecdotal reports describe both immunostimulating and immunosuppressing effects with use of Aloe vera plant components; however, there are few scientifically controlled studies examining these effects. Although there is a general consensus among the studies that the polysaccharide fraction of aloe gel has immunomodulating activities, the identity, size, and composition of the major immunomodulating polysaccharide are not known.

The immunostimulatory properties of commercial preparations of crude whole leaf Aloe vera were evaluated and characterized using a reporter-gene assay (Pugh *et al.*, 2001). A high molecular weight (4-7 x 10^6 Da) polysaccharide fraction, aloeride, induced the expression of mRNAs encoding interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in THP-1 human monocyte cells at levels equivalent to those of cells stimulated by lipopolysaccharide (LPS). The authors suggested that the reported immunomodulatory effects attributed by others to acemmanan were due to the presence of trace amounts of aloeride in the crude juice and aloe gel. In contrast, Qui *et al.* (2000) isolated a much smaller (80 kDa) polysaccharide, modified *Aloe* polysaccharide (MAP), from cellulose-digested aloe gel that was found to activate macrophage cells and stimulate fibroblast growth. The native aloe gel was found to have no effect on macrophage activation. Similarly, fractionated crude cellulose-digested aloe gel was tested for *in vitro* and *in vivo* immunomodulatory activities by Im *et al.* (2005). Polysaccharides between 5- and 400-kDa were found to exhibit the most potent macrophage-activating activity, as determined by increased cytokine production, nitric oxide (NO) release, expression of surface markers, and phagocyte activity. Using differential centrifugation, ion exchange chromatography, and co-cultures of organ slices, Talmadge *et al.* (2004) also purified a high-molecular-weight fraction that showed increased hematological and hematopoietic activity compared with the aloe gel starting material. Increased hematopoietic activity is associated with increased mRNA

levels for hematopoietic cytokines. This profile of activity differed from another purified polysaccharide fraction that had anti-inflammatory activities, suggesting that the conflicting results may be attributable to multiple and potentially conflicting activities of the Aloe vera extracts used in the studies.

Several studies have examined the carbohydrates of Aloe vera gel for macrophage activation as well as the activation of other cell types that function in immune responses. Zhang and Tizard (1996) examined the effects of a commercial preparation of acemannan, alone and in combination with interferon-gamma (INF-γ) on the activation of the murine macrophage-like cell line, Raw 264.7. Acemannan alone could activate the macrophages both directly and indirectly to release IL-6 and TNF-α. Acemannan also synergistically enhanced macrophage sensitivity to IFN-γ as reflected by increased NO release, enhanced surface molecule expression, and cell morphology changes. The same commercial preparation of acemannan was used by Ramamoorthy *et al.* (1996) who demonstrated by northern blot analyses that the acemannan-induced increase in NO production was preceded by increased expression of mRNA for the inducible form of macrophage NO synthase. Furthermore, the induction of NO synthase was inhibited by pre-incubation with pyrrolidine dithiocarbamate, an inhibitor of NFκ, suggesting that acemannan causes the activation of macrophages by increasing the level of NO synthase at the level of transcription. In a subsequent experiment, Ramamoorthy *et al.* (1998) showed that in the presence of IFN-γ, acemannan induced apoptosis in the RAW 264.7 cell line. The cells exhibited typical characteristics of apoptosis, including chromatin condensation, DNA fragmentation, and DNA laddering. Neither acemannan nor IFN-γ induced apoptosis alone; however, the induction of apoptosis appeared to be independent of NO production, since N-nitro-L-arginine methyl ester (L-NAME), a NO inhibitor, did not protect the cells. It was suggested that the induction of apoptosis by acemannan in combination with IFN-γ involved the inhibition of bcl-2 expression.

Other studies have focused on evaluating whether or not the activation of macrophage by acemannan occurs via mannose receptors on the cell surface of macrophage. Karaca *et al.* (1995) demonstrated that normal chicken spleen cells and a chicken bone marrow macrophage cell line, HD11, produced NO and suggested that the acemannan-induced NO synthesis may be mediated through macrophage mannose receptors. In this study, HD11 or isolated chicken spleen cells were treated with serially diluted acemannan, yeast mannan, or LPS. Cells cultured in the

presence and absence of Concanavalin A (Con A) or N-methyl-DL aspartic acid (NMA) were used to evaluate the potential role for mannose receptors. Con A is reported to have a high affinity for terminal mannose residues and may serve to block acemannan. In contrast with results presented by Zang and Tizard (1996), the NO-inducing effect of acemannan alone exhibited a dose-dependent relationship on spleen cells. Similarly, NO production was increased in HD11 cells in response to LPS and to a much lower extent by acemannan, but not to yeast mannan. The failure of yeast mannan to elicit a NO response was explained by involvement of acetylated mannose-specific receptors that may be present in macrophage activation. Con A was shown to inhibit acemannan- and not LPS-induced NO production by HD11 cells in a dose dependent manner; whereas, L-NAME, an inhibitor of NO synthase, inhibited both LPS and acemannan stimulated production of NO.

The release of arachidonic acid and other lipids from tissues and cell membranes results in the formation of lipid peroxides, the generation of free radicals, and the production of prostaglandins. The intragastric administration of an ethanol extract of Aloe vera whole leaves to streptozotocin-induced diabetic rats was shown to reduce lipid peroxidation and the formation of hydroperoxides, and resulted in increased levels of anti-oxidant enzymes, including reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase, in the liver and kidney (Rajasekaran *et al.*, 2005). A juice filtrate of Aloe vera whole leaves administered by gavage to whole body γ-irradiated rats was also shown to reduce lipid peroxidation and improve anti-oxidant enzyme status in the liver, lungs, and kidneys (Saada *et al.*, 2003). Aloe vera was also shown to be effective in minimizing the radiation-induced increase in plasma glucose levels without affecting insulin levels, suggesting that the hypoglycemic effects of Aloe vera may function via decreased hepatic gluconeogenesis. A commercial preparation of aloe gel was shown to inhibit reactive oxygen metabolites and the production of prostaglandins in human colorectal mucosa cells and colorectal biopsies (Langmead *et al.*, 2004). Aloe vera gel, at 1:50 dilution in culture medium, inhibited prostaglandin E_2 production by 30% in inflamed colorectal biopsies, but had no effect at higher or lower concentrations and thromboxane B_2 release was not affected at any dose. The reduced inhibition of prostaglandin E_2 at higher concentrations of aloe gel suggests that one or multiple components in the gel may actually stimulate prostaglandin production and outweigh the inhibitory effects by other components.

In a randomized, double-blind, placebo-controlled trial that examined the efficacy of aloe gel in the treatment of mildly to moderately active ulcerative colitis, patients were administered aloe gel in a drink twice daily for four weeks. Clinical remission, sigmoidoscopic remission, and histological remission were the primary endpoints measured (Langmead *et al.* 2004). The drink was a commercial product of aloe gel and the placebo was a flavored liquid, identical in taste and appearance to the Aloe vera preparation. The physician's global assessment showed no change during the treatment period, and none of the primary end-points of the study were met in terms of clinical, endoscopic, or histologic remission. The Simple Clinical Colitis Activity Index and histological scores decreased significantly; however, the sigmoidoscopic scores and laboratory values showed no significant differences from placebo controls.

TOXICOLOGICAL PROPERTIES OF ALOE VERA GEL

Several studies have attempted to determine whether or not Aloe vera causes toxicity in animals or humans. Various preparations were studied including plant-derived aloe gel, commercial forms of the gel, and isolated components, either commercial or native.

Single or eight repeated 4-day interval doses of a commercial preparation of acemannan were administrated by intravenous or intraperitoneal injections to mice, rats, and dogs (Fogleman *et al.*, 1992a). No signs of treatment-related toxicities were apparent after a single injection of acemannan in mice or rats; however, emesis and diarrhea were observed in dogs following intraperitoneal or intravenous injections. Repeated administration of acemannan was associated with an increased accumulation of macrophages and monocytes in the lungs of intravenously-treated animals and in the liver and spleen of intraperitoneally-treated animals; however, there were no subsequent inflammatory reactions detected after a 6-day recovery period. Clinical signs of intoxication included a decrease in activity, abnormal gait and stance, flaccid body tone, piloerection, and tremors in mice, and emesis, abdominal discomfort, decreased activity, and diarrhea in dogs. Early deaths occurred in 30% of the high (80 mg/kg/dose) dose and 15% of the middle (40 mg/kg/dose) dose mice treated intravenously, and in 25% of the mice dosed intraperitoneally at 100 – 200 mg/kg. Foggleman *et al.* (1992b) also examined the effect of oral administration of a commercial preparation of acemannan in acute and subchronic studies in rats and dogs. Technical grade acemannan

was mixed in the basal diet for the rats or in a canine meal for the dogs. The acemannan was administered to the rats for 14 days at 5% of the diet (approximately 4,000 mg/kg/day) and for 6 months at up to 2,000 mg/kg/day, and acemannan was administered to dogs for 90 days at up to 1,500 mg/kg/day. There were no significant treatment-related effects or mortality in the 14-day study in rats or in the 90-day subchronic study in dogs. In the subchronic rat study, bleeding, enlarged kidneys, and pyelonephritis were observed at necropsy. The technical grade acemannan used in this and the previous study was lyophilized and contained an average of 78-84% acemannan, less than 10% water, and the balance as calcium, magnesium, and other salts.

Acute and chronic toxicity studies were conducted with the ethanolic extract of Aloe vera (Shah *et al.*, 1989). In acute studies, the extract was administered orally at 500 mg/kg, 1 g/kg, and 3 g/kg. The general symptoms of toxicity and mortality were monitored for 24 h. Mice administered the Aloe vera extract by oral gavage showed no acute signs of toxicity at 500 mg/kg during the 24 hour observation period. However, at higher doses (1 and 3 g/kg) a decrease in central nervous activity was noted. During the chronic 90-day study, there was no effect on body and vital organ weights. The Aloe vera leaf extract at a dose of 100 mg/kg in the drinking water induced alopecia of the genital region, and degeneration and putrification of the sex organs were observed in 20% of the animals. Decreased erythrocyte cell counts, a significant spermatogenic dysfunction, and a 30% lethality were significant compared with control animals (Shah *et al.*, 1989).

Nath *et al*. (1992) dosed pregnant rats (5/group) orally for 10 days with 0, 125, 175, 250, 270, and 350 mg/kg of a whole leaf aqueous extract of *Aloe barbadensis* Miller, i.e. Aloe vera. Control animals were dosed orally with the vehicle (1% gum acacia). All animals were laparotomized on day 20 under light anesthetic ether and caesarian delivery was performed. The number of corpora lutea, number of implantations in each horn, correlation of fetal placement in each horn with the number of corpora lutea, total number of fetuses, total number of live/dead fetuses, total number of early/late resorptions, fetal body weight, and fetal body length were noted. Fetuses were placed in 70% alcohol for 24 h and grossly checked for external abnormalities, and, if any were present or suspected, the fetus was processed for skeletal evaluations. In the Aloe vera treatment groups, 51 fetuses were examine for gross abnormalities, 25 fetuses were examined for visceral abnormalities, and 26 for skeletal abnormalities. Macroscopic findings included kinking of tail, clubbing of right hind limb, and left wrist drop. There were no visceral

abnormalities observed; however, skeletal abnormalities included a 15.4% incidence, with wavy ribs, non-osified ribs, tarsal fused, and intercostal space in ribs the prominent skeletal abnormalities. In the low dose animals (125 mg Aloe vera/kg body weight) on a per animal basis, implantations, resorptions, and live births were higher than controls, while fetal body weights and body lengths were lower. Overall a 21.5% abortifacient activity (resorptions/implantations) was calculated compared with 0.0% in controls.

Largarto Parra *et al*. (2001) administered plant extracts, including Aloe vera (Barbados aloe) orally to Swiss albino mice (number of animals not provided) for an estimation of the LD_{50}. The Aloe vera leaves were dried and chopped into particles (≤ 5 mm), and the fluid extracts were obtained by percolation with 4 alcoholic extractions. The ratio of solvent volume to the weight of the plant material was 3:1. For every plant extract, three concentrations (in triplicate) were tested in order to determine dose-response relationships, and a control group was administered the vehicle used for the dilutions of the extracts. The LD_{50} values were estimated using mortality results obtained 24 h after oral administration. The Aloe vera fluid extract (LD_{50} = 120.65 mg/kg) was the most toxic of the 21 plants tested. *In vitro* tests in brine shrimp larva were in agreement with the *in vivo* toxicity tests in mice.

The effect of ingestion of crude and decolorized Aloe vera gel on growth, dietary intake, and a variety of metabolic parameters in rats was examined in 1.5 and 5.5 month studies (Herlihy *et al.*, 1998a; 1998b). Aloe vera gel was prepared by two methods and mixed with rat chow at selected concentrations. Crude Aloe vera gel was prepared from skinned Aloe vera leaf filets by homogenization followed by lyophilization and grinding to a fine powder, decolorized Aloe vera gel was prepared similarly except that the homogenate was decolorized by charcoal filtration prior to lyophilization. Ingestion of the crude Aloe vera gel produced diarrhea, a slower growth, polydipsia, and polyuria in rats compared with control animals at concentrations of 3%, 5%, and 10% of the diet (approximately equivalent to 330, 550, and 1,100 mg/kg/day). At a dietary concentration of 1% (approximately 110 mg/kg/day), neither the crude nor the decolorized Aloe vera gels elicited adverse effects on growth or pathology. The dietary ingestion of the crude or decolorized Aloe vera gel for 5.5 months by rats resulted in marked changes in serum parathyroid hormone and calcitonin concentrations, suggesting that Aloe vera gel may alter calcium metabolism (Herlihy *et al.*, 1998b).

The effects of lifetime administration of dietary-supplemented Aloe vera crude gel, decolorized Aloe vera gel, or decolorized Aloe vera whole leaf extract was examined in rats (Ikeno *et al.*, 2002). Commercial preparations of Aloe vera crude gel and decolorized Aloe vera gel were incorporated into a semi-purified diet at 1% (wt/wt) and administered *ad libitum*. The Aloe vera decolorized whole leaf was administered at 0.02% (wt/vol) in the drinking water of rats. In general, the life-long ingestion of Aloe vera exerted no apparent harmful effects or changes in physiological parameters in the rat.

Lim *et al.* (2003) used an almost identical protocol of administration to examine supplementation of rats with Aloe vera on anti-oxidant protection and cholesterol-lowering effects. Groups of male Fischer 344 rats (5 rats/group) were fed diets without Aloe vera supplementation, diets containing 1% (wt/wt) freeze-dried Aloe vera crude gel or 1% decolorized freeze-dried Aloe vera gel, or were fed the unsupplemented diet and administered 0.02% (wt/wt) freeze-dried Aloe vera decolorized whole leaf in the drinking water. Significantly reduced hepatic phosphatidylcholine hydroperoxide levels were observed in Aloe vera supplemented groups at 4 months compared with control animals. Dietary Aloe vera administration significantly enhanced catalase and superoxide dismutase levels, but the supplementation of Aloe vera in the drinking water had no effect. Total cholesterol levels were not different from control levels at 4 months, but Aloe vera supplementation significantly lowered cholesterol levels in 16 month old rats (Lim *et al.*, 2003).

A clinical case report was presented of a female patient with a 1-week history of progressive jaundice, pruritus, alcoholic bowel movements, and abdominal discomfort, who began ingesting tablets of an unspecified extract of *Aloe barbadensis* Miller (500 mg/tablet) 4 weeks prior to admission (Rabe *et al.*, 2005). Liver biopsy revealed severe acute hepatitis with portal and acinar infiltrates of lymphocytes, plasma cells, granulocytes along with bridging necrosis and bilirubinostasis. The hepatitis was linked to the ingestion of Aloe vera tablets, and symptoms resolved upon discontinuance within 1 week.

There is potential for herb-drug interactions with Aloe vera components in patients using prescribed mediations. Compounds in Aloe vera may cause a reduction in prostaglandin synthesis, which may inhibit secondary aggregation of platelets. Vasquez *et al.* (Vázquez *et al.*, 1996) showed that aloe gel caused a 48% reduction in

prostaglandin synthesis compared with a 63% reduction by indomethacin. A case was presented in which a female patient lost 5 liters of blood during surgery as a result of a possible herb-drug interaction between oral consumption of Aloe vera tablets and sevoflurane, an inhibitor of thromboxane A_2 (Rabe *et al.*, 2005). Interactions of aloe gel have also been reported for hydrocortisone, antidiabetic agents, and UV radiation (Mascolo *et al.*, 2004).

BIOLOGICAL PROPERTIES OF ALOE VERA LATEX

Metabolism

Aloe vera latex contains a mixture of anthracene compounds including O- and C-glycosides of anthrones and anthraquinones, as well as free anthrones and dianthrones and a small amount of free anthraquinones (Brusick and Mengs, 1997). Orally ingested anthranoid glycosides pass through the upper part of the gastrointestinal tract without chemical modification. The sugar moiety confers hydrophilic characteristics to the anthraquinone glycoside, which prohibits absorption by intestinal epithelial cells. This results in the passage of anthraquinone glycosides to the lower gastrointestinal tract and colon unmodified, where resident microflora of the *Bifidobacterium* sp. catabolize the *O*-glycosidic anthranoids, while bacterium of the *Eubacterium* sp. act upon the C-glycoside anthranoids, to release the sugar moiety and the free anthraquinone aglycone (Hattori *et al.*, 1993; van Gorkom *et al.*, 1999). The laxative activity of the Aloe vera latex is not due to the ingested form of the anthraquinone, but rather to a common metabolite, aloe-emodin-9-anthrone (Figure 2), which is formed by activity of the *Eubacterium* BAR (Che *et al.*, 1991; Hattori *et al.*, 1993; Akao *et al.*, 1996). The *Eubacterium* sp. is expressed differentially across mammalian species; for example, rats and not guinea pigs are able to generate the aloe-emodin-9-anthrone (deWitte, 1993). Subsequent systemic metabolism of the free anthranoids depends upon their absorption and ring constituents (Sendelbach, 1989). Free anthraquinone aglycones undergo oxidation to form anthrones and anthraquinones that are absorbed through the small intestine, where they are transported to the liver and glucuronidated (Stolk and Hoogtanders, 1999). The glucuronidated compounds are partially excreted in the urine with the remainder returned to the intestine through the bile (Sendelbach, 1989). The glucuronidated anthraquinones are transported to the colon and released as free anthraquinones after metabolism by gut bacterial enzymes (deWitte and Lemli, 1990; deWitte, 1993). Most of the free anthranoids absorbed systemically in humans are excreted in the urine as rhein (Figure 2) or as conjugates (Vyth and Kamp, 1979; deWitte and Lemli, 1990; deWitte, 1993).

Barbaloin was dissolved in distilled water initially at 20 mg/ml and administered orally to male Wistar rats at a dose of 100 mg/kg (Ishii *et al.*, 1987). At defined times after the administration of barbaloin, blood was collected from the carotid artery of rats to measure serum levels of barbaloin. Barbaloin was first observed in serum at 30 min after administration (0.092 μg/ml) and the maximum concentration (0.337 μg/ml) was reached at 90 min. Serum levels of barbaloin decreased for up to 3 h, but were still detectable at 6 h post administration. The authors offered three possibilities for the extremely low serum concentrations of barbaloin as either low absorbability from the gastrointestinal tract of rats, high degradability by rat gastrointestinal microflora, or high transferability of barbaloin from rat serum to tissues (Ishii *et al.*, 1987).

The administration of barbaloin (31.5 mg/5 ml/kg) in a 5% gum arabic solution by cecal intubation to male Wistar rats produced aloe-emodin-9-anthrone in the rat large intestine and caused not only an increase in the intestinal water content but also stimulated mucus secretion. Aloe-emodin-9-anthrone peaked at 4 h after administration of barbaloin and was detected in the cecum at 508 μg/rat and in the colon at 83 μg/rat. Diarrhea was observed in all rats by 9 h; however, normal feces were still excreted by some rats at 8 h. Barbaloin required several hours for its activation to aloe-emodin-9-anthrone, even after intracecal administration, as activation depended upon the activity of intestinal bacteria (Ishii *et al.*, 1994).

The ability of free anthraquinones to be absorbed in the small intestine appears to determine their toxic potential (Sendelbach, 1989). Lang (1993) administered ^{14}C-aloe-emodin to male and female SPF Brown-Norway rats orally in a tragacanth (0.3%) suspension at a dose of 4.5 mg/kg. Blood, feces, urine and organs were collected at specified time points to elucidate the distribution of the compound. Results showed that 20%-30% of the dose was excreted in the urine and the rest was excreted in the feces as rhein and conjugates. Ten percent of the radioactivity was identified as free aloe-emodin in the plasma, with maximum concentrations (248 ng equivalents aloe-emodin/ml in males and 441 ng equivalents aloe-emodin/ml in females) peaking at 1.5-3.0 h post administration. Maximum plasma levels were about three and ten times higher than the concentrations in the ovaries and testes, respectively. Only the liver, kidney, and intestinal tract showed higher concentrations than the plasma. The terminal half-life of the radioactivity in the blood was 50 h (Lang, 1993).

The kinetic dynamics of aloe-emodin and rhein were determined after administering therapeutic doses of senna laxatives orally to 10 healthy volunteers in a two-way cross-over design. Blood samples were collected up to 96 h after the first dose, and plasma levels of total aloe-emodin and rhein were determined by fluorometric HPLC. Aloe-emodin was not detectable in any plasma sample of any subject. The concentration of rhein showed the highest level at 3-5 h and another peak maxima at 10-11 h after dosing, which were probably dependent upon the absorption of free rhein and rhein released from the pro-drugs (e.g. sennosides) by bacterial metabolism, respectively(Krumbiegel and Schulz, 1993).

Cathartic Effects

Aloe vera latex possesses laxative properties, and use of the latex to relieve constipation dates back to classic Greece with first recordings of its use in the first century A.D. (Fantus, 1922). In general, diarrhea is induced by an increase in water content and/or peristalsis in the large intestine. The major C-glycosides of Aloe vera latex, barbaloin and isobarbaloin (Figure 2), are the principal agents responsible for the cathartic activities of Aloe vera in humans and animals, although considerable variation exists in purgative potency among animal species; for example, barbaloin is potent in humans but shows reduced activity in the mouse and rat (Hattori *et al.*, 1988; Che *et al.*, 1991; Joshi, 1998). In addition, there are inter-individual differences in sensitivity to the laxative activity of barbaloin (Ishii *et al.*, 1993). Both barbaloin and isobarbaloin are inactive as laxatives themselves but undergo decomposition to form aloe-emodin-9-anthrone (Figure 2) and aloe-emodin and other metabolites by human and animal intestinal flora (Hattori *et al.*, 1988; Ishii *et al.*, 1990; Ishii *et al.*, 1998). The human intestinal anaerobe, *Eubacterium* BAR, was shown to metabolize barbaloin and induce severe diarrhea in gnotobiotic rats (Che *et al.*, 1991; Hattori *et al.*, 1993; Akao *et al.*, 1996). Diet and nutrition were also shown to play important roles in the laxative action of aloe latex. The metabolism of barbaloin to aloe-emodin-9-anthrone was promoted by a diet containing iron salts and iron-rich meat and was decreased by cereals and complex carbohydrates (Koch, 1996). In addition, individual anthrones exhibit less purgative activity than mixtures of anthrones or of mixtures of anthrones and anthraquinones, suggesting that metabolites of barbaloin synergistically exert purgative effects (Yagi and Yamauchi, 1999).

Confirmation of aloe-emodin-9-anthrone as the purgative principle of Aloe vera latex was demonstrated by the intracecal administration of barbaloin and subsequent detection of aloe-emodin-9-anthrone in the large intestine, with accompanying diarrhea (Ishii *et al.*, 1994). The aloe-emodin-9-anthrone and anthraquinones of barbaloin and

isobarbaloin are thought to utilize multiple mechanisms in producing their cathartic effects. *In vitro* and *in vivo* studies in rats demonstrated that aloe-emodin-9-anthrone disturbs the equilibrium between the absorption of water from the intestinal lumen via inhibition of active sodium/potassium-adenosine triphosphatase and increases the paracellular permeability across the colonic mucosa (Ishii *et al.*, 1990), stimulates peristaltic activity in the large intestine, stimulates mucus secretion(Ishii *et al.*, 1994), and secretes water into the lumen by a prostaglandin-dependent mechanism (Capasso *et al.*, 1983). The result is a net reduction in water absorption and more frequent stools with softer consistency. Aloe-emodin-9-anthrone was shown to enhance the membrane permeability of water-soluble and poorly permeable compounds in the rat colon (Kai *et al.*, 2002). The permeation-enhancing activity was estimated by changes in the permeability coefficient of 5(6)-carboxyfluorescein, and aloe-emodin-9-anthrone was shown to significantly increase its permeation in a dose-dependent manner. The enhancing effects were inhibited by an inhibitor of protein kinase C and significantly suppressed by a histamine H_1 receptor antagonist and a mast cell stabilizer. The results suggest that aloe-emodin-9-anthrone stimulates colonic mast cells to release histamine, which activates the protein kinase C pathway and opens tight junctions in colonic membranes.

Although there is no doubt that Aloe vera latex exerts its action on the colonic mucosa, its mechanism of action is still not fully understood. Under physiological conditions, endogenous NO appears to function as a pro-absorptive molecule, based on findings that NO synthetase inhibitors reverse net fluid absorption to net secretion in rodents, dogs, and rabbits (Izzo *et al.*, 1998). When rats were treated with several laxatives, including castor oil and anthraquinones of senna and cascara, NO was elevated in their colon, and L-NAME, a NO synthetase inhibitor, reduced their diarrhea response (Izzo *et al.*, 1998). L-NAME was also shown to prevent the diarrhea and fecal water excretion in rats administered aloe or barbaloin; however, in contrast with castor and senna laxatives, aloe and barbaloin produced a dose-dependent inhibition of calcium-dependent NO synthase activity in the rat colon, suggesting that the inhibition of NO synthetase by aloe or barbaloin may be a mechanism to reduce the cathartic activity of aloe (Izzo *et al.*, 1999). Aloe-emodin was also shown to inhibit the autotoxic release of NO in a dose-dependent manner in murine L929 fibrosarcoma cells that were stimulated with interferon-gamma and interleukin-1 (Mijatovic *et al.*, 2004).

Anti-bacterial/Anti-viral Activity

The phenolics and aloins of Aloe vera were found to have dose-dependent non-competitive inhibitory effects on *Clostridium histolyticum* metalloproteinases and collagenases (Barrantes and Guinea 2003). Structure activity relationships drawn between the aloins and tetracyclines suggest that the inhibitory effects of aloins are via a destabilizing effect on the structure of the granulocyte metalloproteinases and diminishing intracellular calcium availability ('tHart *et al.* 1990). Barbaloin was also shown to disrupt membranes by weakening hydrophobic interactions between hydrocarbon chains in the phospholipids bilayers. Moreover, barbaloin showed specificity for two major phospholipids (phosphatidylethanolamine and phosphatidylglycerol) present in bacterial membranes (Alves *et al.* 2004). In screenings of Aloe vera for anti-viral effects, aloe emodin purified from barbaloin, was also shown to inactivate a variety of viruses, including herpes simplex virus type I and type II, varicella-zoster, and the influenza virus (Sydiskis *et al.* 1991). In tests of barbaloin to inhibit the infectivity of the viral hemorrhagic septicemia rhabdovirus or the growth of *Escherichia coli*, barbaloin exhibited anti-viral but not virucidal activity (Alves *et al.* 2004). Others reported differing results (Anderson *et al.* 1991). The mechanism proposed for the anti-bacterial and anti-viral effects of aloe-emodin is the inhibition of nucleic acid biosynthesis after which protein syntheses is also inhibited (Levin *et al.* 1988). The tetracyclines are also able to inhibit protein synthesis at the ribosome level, probably by interference with the ribosome messenger and RNA, and perhaps aloe-emodin acts similarly (Friedmann 1980).

Anti-oxidant/Pro-oxidant Activity

The anti-oxidant activities of anthraquinone and anthrones of Aloe vera have been evaluated using different model systems (Hutter *et al.*, 1996; Lee *et al.*, 2000; Yen *et al.*, 2000). An aloesin derivative from Aloe vera was found to exhibit potent anti-oxidant activity and inhibit cyclooxygenase-2 and thromboxane A2 synthase. Aloe-emodin was also shown to have some protective effects against carbon tetrachloride-induced lipid peroxidation in rat liver (Arosio *et al.*, 2000). Aloe emodin not only protected against hepatocyte death but also protected against the inflammatory response subsequent to lipid peroxidation.

Anthraquinone and anthrones of Aloe vera absorb UV light in the UV-B range. *In vitro* studies on the photobiological and photochemical properties of barbaloin and aloe-emodin were conducted in human skin fibroblasts (Wamer *et al.*, 2003). Cells were incubated with barbaloin or aloe-emodin and exposed to UV or visible

light. Cells pretreated with aloe-emodin showed increased sensitivity to both UV-A and visible light. Significant photo-oxidative damage to both RNA and DNA was associated with the phototoxicity induced by aloe-emodin. Oxidative damage was observed even at low levels of phototoxicity, which suggested that photo-oxidative damage may cause rather than result from cellular death induced by aloe-emodin. The phototoxicity mechanism for aloe-emodin appears to involve the generation of reactive oxygen species and stable photoproducts with cellular components (Vargas *et al.*, 2002). Aloe-emodin was found to generate singlet oxygen efficiently when irradiated with UV light, and the survival of human skin fibroblast in the presence of aloe-emodin was found to decrease when irradiated (Vath *et al.*, 2002).

Cytotoxicity/Anti-tumoral Effects

Aloe vera, in general, and aloe-emodin, in specific, has been reported to have *in vitro* cytotoxic effects against tumor and not normal cells. Aloe-emodin was shown to have specific dose-dependent cytotoxic effects on non-epithelial tumors, in particular neuroblastoma cells; however, human epithelial tumors, blood-derived tumors, and normal fibroblasts were almost refractory to the aloe-emodin treatments (Pecere *et al.*, 2000). In addition, of five purified anthraquinone compounds isolated from Aloe vera, only aloe-emodin produced cytotoxic effects against the multi-drug resistant human leukemia cells, although the effective dose range was in the micromolar concentration range (Grimaudo *et al.*, 1997). The aloin glycosides, aloesin, and aloeresin were devoid of anti-tumor cell activity, implying that only aloe-emodin exerted cytotoxic responses. Treatment of human leukemia cells with aloe-emodin was shown to induce cell cycle arrest, with the subsequent accumulation of cells in the S and G_2-M phases of the cell cycle, and at increased doses aloe-emodin was also shown to induce apoptosis in human lung squamous carcinoma cells (Chen *et al.*, 2004). Subsequently, it was demonstrated that the mechanism of aloe-emodin induced apoptosis involved the modulation of the expression of Bcl-2 family proteins, activation of caspases, and decreased the expression of certain isozymes of protein kinase C, suggesting that aloe-emodin induced apoptosis occurred via activation of the Bax and Fas pathway (Lee *et al.*, 2001a; Lee *et al.*, 2001b). The expression of p38 may also be an important determinant of apoptotic death induced by aloe-emodin (Yeh *et al.*, 2003). The exposure of aloe-emodin to two liver cancer cell lines that differed in p53 expression, however, suggested alternative mechanisms for the differing anti-proliferative activities of aloe-emodin. In human liver cancer cells that express p53, aloe emodin induced a p53-dependent pathway that was accompanied with enhanced expression of p21 and resulted in cell cycle arrest. In human liver cancer cells that were p-53 deficient, aloe-emodin was shown to induce a p21-dependent

pathway that did not cause cell cycle arrest, but rather promoted apoptosis (Kuo *et al.*, 2002). In cell-based ELISA and Western blot analysis, aloe-emodin was shown to abolish cisplatin-triggered activation of extracellular signal-regulated kinase (ERK) in rat glioma and murine fibrosarcoma cells (Mijatovic *et al.*, 2005).

Shimpo *et al*. (2001) examined the modifying effects of a whole-leaf extract of *Aloe arborescens* Miller, which is a different species of Aloe than Aloe vera, on azoxymethane-induced aberrant crypt foci in the rat colorectum. Male F344 rats were fed basal diet or experimental diets containing 1.0 or 5.0% aloe for 5 weeks. One week later, all rats, with the exception of vehicle controls, were injected subcutaneously with azoxymethane (15 mg/kg, once weekly for 3 weeks). At 9 weeks of age, rats were sacrificed and the colorectum and liver were evaluated for aberrant crypt foci and cytosolic quinine reductase. In rats administered the *Aloe arborescens* Miller (1.0 or 5.0%) and azoxymethane, the numbers of aberrant crypt foci were significantly decreased compared with rats that received azoxymethane alone. Rats that were administered *Aloe arborescens* Miller had significantly increased cytosolic quinine reductase activity in the liver, suggesting that *Aloe arborescens* Miller might have a chemopreventive effect against colon carcinogenesis in the initiation stage (Shimpo *et al.*, 2001).

Subsequently, the modifying effect of freeze-dried whole leaf *Aloe arborescens* Miller on azoxymethane-induced intestinal carcinogenesis was examined in F344 rats (Shimpo *et al.*, 2006). Male F344 rats were fed basal diet or experimental diet containing 0.2 or 1.0% Aloe for 28 weeks. Two weeks after initiation of the diets, the animals received subcutaneous injections of azoxymethane once weekly for 10 weeks. The incidence of colorectal adenocarcinomas in the 0.2%, but not the 1.0%, aloe group showed a tendency of decrease ($P = 0.056$) from the control group. The incidence of adenocarcinoma in the entire intestinal tract (small and large intestine) in the 0.2% aloe group was significantly ($P = 0.024$) decreased compared to control levels. There were no significant differences in tumor multiplicities of colorectal or intestinal among the three groups (Shimpo *et al.*, 2006).

TOXICOLOGICAL PROPERTIES OF ALOE VERA LATEX

Aloe vera latex contains many biologically active compounds, but it usually taken as a purgative (Mapp and McCarthy, 1970). Tumor-promoting as well as anti-mutagenic activities have been ascribed to the latex of Aloe vera. Mutagenic and genotoxic activities in bacteria and eukaryotic cells have been shown for some, but not all

anthraquinones. Westendorf and coworkers (1990) investigated naturally occurring hydroxyanthraquinones for mutagenicity and cell-transforming activity. Aloe-emodin, which is present in Aloe vera-anthraquinoid laxatives, exhibited dose-related effects in mutation assays, in rat hepatocyte DNA-repair induction assays, and in assays to determine malignant transformation of C3H/M mouse fibroblasts. Mueller and colleagues (1996) investigated the genotoxicities of several anthraquinone derivatives found as natural constituents in plants and showed that some of the 1,8-dihydroxyanthraquinone derivatives, including aloe-emodin are intercalating agents that inhibit the interaction between topoisomerase II and DNA. The compounds induced a moderate increase in *Tk*-mutations and a dose-dependent induction of micronuclei. A micronuclei test indicated that danthron was more potent than aloe-emodin, which was more potent than emodin. Kodama and associates (1987) observed DNA strand breaks and the generation of free radical and hydrogen peroxide by some anthraquinone derivatives from plant sources; and, subsequently, Mueller *et al.* (1998a; 1998b; 1999) showed that some anthraquinone derivatives are biotransformed by cytochrome P450 1A2 *in vitro* and that this may be relevant for the disposition of anthraquinone derivatives *in vivo*.

Aloe-emodin and other dihydroxyanthraquinones were examined for activities associated with tumor promotion, such as stimulation of cell proliferation and enhancement of malignant transformation (1990). The *in vivo* treatment of primary rat hepatocytes with danthron, aloe-emodin, chrysophanol, and rhein resulted in a 2-3-fold increase of DNA synthesis, whereas emodin was essentially inactive. This marked stimulation of DNA synthesis was in the range with other known *in vitro* tumor promoters, such as phenobarbital and hexachlorocyclohexane. The results suggested that anthraquinones that possess hydroxyl groups in two positions may have tumor promoting activities. Muller *et al.* (1996; 1999) investigated the dihydroxyanthraquinones of emodin, danthron, and aloe-emodin for genotoxicity in a number of *in vitro* assays, including mutation and micronucleus assays in mouse L5178Y cells, kinetochore analysis, topoisomerase II assay, and comet assays. Emodin, danthron and aloe-emodin reduced the amount of monomer DNA generated by topoisomerase II, indicating that all three compounds were capable of inhibiting the topoisomerase II-mediated decatenation. Furthermore, a modified comet assay showed that pretreatment of the cells with the test compounds reduced the effects of etoposide, an inhibitor of topoisomerase II. Danthron and aloe-emodin, and not emodin increased the fraction of DNA moving into comet tails at concentrations

of 50 μM in single-cell gel-electrophoresis assays. Results of these assays indicate that danthron and aloe-emodin are genotoxic.

SW480 colorectal tumor cells, VACO235 adenoma cells, and normal colonic epithelial cells were exposed to the dihydroxyanthraquinone compounds (0.2-5 mg/ml) of laxatives to determine if these compounds stimulated growth and the secretion of urokinase (1998). Concentrations of 5 mg/ml caused between 50%-70% cell loss in colorectal carcinoma SW480 cells; however, DNA synthesis was not similarly reduced. Dihydroxyanthraquinone treatment caused an approximate doubling in the number of premalignant VACO235 cells; whereas, the growth of normal rat colonic epithelial cells was not affected. Urokinase secretion was increased by all dihydroxyanthraquinones in a dose-dependent manner, and this was the predominant effect of the dihydroxyanthraquinones in the SW480 carcinoma cells. Urokinase facilitates metastasis by matrix degradation and digestion of normal cells, and it was suggested that the release of urokinase caused the loss of cells observed in the SW480 carcinoma line.

Four *in vivo* studies were conducted to investigate the genotoxicity of aloe-emodin and emodin (Brusick and Mengs, 1997). The studies were conducted in rats or mice orally administered aloe-emodin or emodin for 4 h to 9 days of duration. Analyses were conducted on bone marrow cells by micronucleus testing or in mouse fetal melanoblasts with the mouse spot test. The results showed no evidence of compound-induced increases of micronuclei or evidence of mutation induction or clastogenicity, although blood concentrations of aloe-emodin in the animals reached levels in the range of genetically active concentrations *in vitro*. One area of testing that was not addressed is the potential for effects in the gastrointestinal tract where the concentrations would be higher and where the microflora environment may actively participate in the metabolism of these compounds.

Matsuda *et al*. (2008) conducted a 1 year pilot study to evaluate the chronic toxicity of *Aloe arborescens* Miller in the diet at doses of 0.16, 0.8, and 4.0% to groups of male and female Wistar Hannover rats. No deaths occurred at any dose level throughout the treatment period. Diarrhea and reduced body weight gains were observed in both sexes of rats that received the 4.0% diet. Changes in hematological parameters were observed in male and female rats, especially at the 4.0% diet level. Relative kidney weights were increased in the 4.0% female group, and relative heart and brain weights were decreased in the 0.8 and 4.0% female groups. Histopathologically, both sexes

receiving the 4.0% aloe showed severe sinus dilatation of the ileocecal lymph nodes and pigmentation of the ileocecal lymph nodes and renal tubules. No other test substance-related changes were observed (Matsuda *et al.*, 2008).

A 2-year carcinogenicity study of Aloe, *Aloe arborescens* Miller, was conducted for assessment of toxicity and carcinogenic potential in the diet at doses of 0.8 or 4.0% in groups of male and female Wistar Hannover rats (Yokohira *et al.*, 2009). The whole leaf powder of *Aloe arborescens*, the same grade used as a food additive, was mixed at concentrations of 0.0 (Control), 0.8, and 4.0% into powdered basal diet and pelleted. The concentrations of aloenin and aloin (barbaloin and isobarbaloin) in the whole leaf powder of *Aloe arborescens* and the pelleted diet were measured and evaluated using high-performance liquid chromatography. The concentrations of aloin and aloenin in the whole leaf powder after storage for 2 weeks were 0.83 and 1.91%, respectively. The concentrations of aloin and aloenin in the pelleted diet after 2 weeks of storage at room temperature were 0.0009 and 0.0022% for the 0.8% diet and 0.0179 and 0.0663% for the 4.0% diet. Both sexes receiving the 4% concentration showed diarrhea, with loss of body weight gain. The effects were more prominent in male rats. No other obvious findings were observed, and feed consumptions showed no significant changes in any group. Relative weights of the liver and absolute and relative spleen weights were increased in males, and relative uterine weights were significantly increased in females. Results of hematology and clinical chemistry showed some slight changes in parameters, but no dose response. Microscopically, in male and female 4.0% groups, some ileocecal lymph nodes appeared swollen. The incidences of severe dilatation of the mesenteric lymph sinus were significantly elevated as compared with controls. A significantly elevated incidence in the thickening of colonic epithelium was also found in the 4.0 and 0.8% male and the 4.0% female groups. In the cecum, colon and rectum, adenomas and adenocarcinomas were significantly more frequent in 4.0% males than in controls (Yokohira *et al.*, 2009).

Adverse effects resulting from ingestion of the Aloe vera latex have been reported. Aloe vera latex possesses laxative properties and has been used traditionally to treat constipation. The glycoside anthraquinones are chemically stable in the stomach and, the sugar moiety prevents their absorption into the upper gastrointestinal tract and subsequent detoxification in the liver. Once they reach the large intestine, the glycoside anthraquinones act like pro-drugs, and bacterial glycosidases liberate the aglycones, such as aloe-emodin. The aglycocones evoke secretory

and motility changes in the colon. Prolonged use is associated with watery diarrhea leading to electrolyte imbalance, and an increased loss of potassium that can lead to hypokalemia (Cooke, 1981). The loss of potassium can vary between 25% and 50% of the lean body mass (Heizer *et al.*, 1968). The increased loss of potassium is largely the result of compensatory reaction to the excessive loss of sodium from increased levels of intraluminal prostaglandin E_2 and muscosal cyclic adenosine 3:5-monophosphate, which induces a compensatory production of aldosterone that can exacerbate the hypokalemic condition and increase rennin production (Mascolo *et al.*, 2004). Ishii *et al.*(1990) demonstrated that aloe-emodin-9-anthrone inhibited rat colonic sodium-potassium adenosine triphosphatase. Persistent hypokalemia can result in renal tubular nephropathy and an increased risk to pyelonephritis (Perkins *et al.*, 1950). In a case report, a male patient, who ten days prior to clinical admission had consumed the juice extracted from four to five leafs of Aloe vera, presented with severe arthralgias, palpable purpura, and abdominal pain (Evangelos *et al.*, 2005). The patient had consumed the same remedy 2 months prior without incidence. Within 24 h of the last consumption, a rash on his legs and a mild arthralgia on his ankle were noted. His symptoms worsened in the following days with symmetrical arthralgias involving his knees, elbows, wrists, and ankles. Urinalysis showed hematuria, leukocytes, and moderate proteinuria. A diagnosis of Henoch-Schonlein, which is a systemic vasculitis, was confirmed by skin biopsy. Renal function deteriorated, and a renal biopsy demonstrated segmental necrosis. The immunomodulatory therapy response was poor, and the patient succumbed to renal failure. The renal dysfunction, nephritis, and chronic renal failure have been associated with *Aloe* consumption (Luyckx *et al.*, 2002).

The increased loss of potassium may potentiate the actions of conventional drugs, such as cardiac glycosides and corticosteroids. Such interactions may result in cardiac arrhythmias and hypertension (Abebe, 2003; Mascolo *et al.*, 2004). In addition, possible antagonism may also occur for anti-diarrhea agents and for non-steroidal anti-inflammatory agents; whereas synergism or exacerbation may result from interactions with glucoresins and diuretics. A decreased gastrointestinal transit time may also reduce the absorption of essential nutrients and many other drugs taken orally.

In recent years the risk of development of colon cancer has been correlated with constipation and the use of laxatives. Apart from the physical changes, such as increased motility and the secretion of fluid and electrolytes

within the lumen, morphological changes induced by laxative use is decidedly of greater importance (Cooke, 1981). Siegers *et al.* (1993) evaluated the incidence of colorectal cancer and anthranoid laxative abuse in humans, using the presence of pseudo-melanosis coli as an indicator of anthranoid abuse. In a retrospective study of 3049 patients who underwent diagnostic colorectal endoscopy, the incidence of pseudo-melanosis coli in patients without pathological changes was 3.1%; the incidence increased significantly to 8.6% in those diagnosed with adenomas, and was 3.3% in patients diagnosed with colorectal carcinomas. In a prospective study of 1095 patients, the incidence was 6.9% for patients with normal diagnoses. The incidence of pseudo-melanosis coli increased to 9.8% for patients with adenomas and 18.6% for patients with carcinomas, suggesting an increased relative risk for colorectal cancer. Although the intestinal absorption and expected concentrations of 1, 8-dihydroxyanthraquinones in human tissues by food intake or medications is low, local accumulation is possible and the relative increased risk of colorectal cancer among frequent laxative users suggests that further research is warranted.

The onset of colonic lesions was examined in a patient who underwent liver transplantation and was also known to suffer from ulcerative colitis (Willems *et al.*, 2003). A medical history of the patient revealed a 10 month use of an aloe-containing anthranoid laxative. Colonoscopy showed marked brownish pigmentation of the mucosa of the entire colon, compatible with melanosis coli; whereas, previous colonoscopies revealed no abnormalities. A year later, a large sessile polypoid lesion was found in the traverse colon, and histological examination revealed tubulovillous adenoma with extensive low-grade dysplasia.

The relationship between sigmoid cancer, constipation, anthranoid laxative use, and melanosis coli was investigated using aberrant crypt foci analysis. Fifty-five surgical patients with sigmoid cancer, 41 surgical patients with diverticular disease, and 96 age- and sex-matched subjects without intestinal disease were interviewed on their history of constipation and anthranoid laxative use. Melanosis coli and aberrant crypt foci characteristics were investigated on sigmoid mucosa of patients with sigmoid cancer or diverticular disease. Constipation and anthranoid laxative use were similar between patients with sigmoid cancer (30.9 and 32.7%, respectively) and those with diverticular disease (39 and 26.8%, respectively) but were higher than among controls (18.8 and 8.3%). The frequency of aberrant crypt foci was higher in patients with sigmoid cancer than those with diverticular disease, and it did not vary with constipation, laxative use, or melanosis coli in either group. There was a positive association of

aberrant crypt foci frequency with colon cancer, but there was no cause-effect relationship of colorectal cancer with constipation, anthranoid laxative use, or with melanosis coli (Nascimbeni *et al.*, 2002).

STUDY RATIONALE

It is estimated that 38% of the U.S. adult population rely on herbal remedies for both general health promotion and the specific treatment of ailments (Wadman, 2009). Aloe vera, a frequently used synonym for the *Aloe barbadensis* Miller plant, has enjoyed a long history of lay acceptance as an herbal remedy and is perhaps the most popular herbal remedy in use today (Klepser *et al.*, 2000; Vogelzang, 2001).

The National Cancer Institute nominated Aloe vera, as a widely used dietary supplement, for studies by the National Toxicology Program (NTP) because of the potential widespread human exposure to adults, children, infants, and the elderly and because studies suggested that components in Aloe vera may possess tumor-promoting activities.

The National Center for Toxicological Research (NCTR) conducted 14-day, 13-week, and 2-year carcinogenesis studies on the leaf extracts of Aloe vera plants. The Aloe vera plant extracts used in these studies were obtained from freshly harvested *Aloe barbadensis* Miller plants and were freeze-dried (6% moisture) and gamma-irradiated to preserve quality. No other additives were used in their preparation. Drinking water was the selected route of administration, because Aloe vera products are consumed in liquid form by the public. This is the first systematic study to examine the safety and carcinogenic potential of Aloe vera plant extracts administered to F344/N rats and B6C3F_1 mice in the drinking water for 2 years.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF ALOE VERA EXTRACTS

The Aloe vera leaf extracts used in these studies were from *Aloe barbadensis* Miller plants that were cultivated in Harlingen, Texas. Leaf weights were a minimum of 400 grams at harvest, and the time from harvest to lyophilization was a maximum of 6 h. The lyophilized (max. 6% moisture content) Aloe vera leaf extracts used in the 14-day, 13-week, and 2-year studies were obtained from Pangea Phytoceuticals, Inc. (Harlingen, TX). For the 14-day studies, extracts included *Aloe barbadensis* Miller Process A gel (Aloe vera gel), *Aloe barbadensis* Miller non-decolorized whole leaf (Aloe vera whole leaf), and *Aloe barbadensis* Miller decolorized whole leaf (Aloe vera decolorized whole leaf) extracts. The 13-week and 2-year studies used only the *Aloe barbadensis* Miller nondecolorized whole leaf (Aloe vera whole leaf) extract.

The Aloe vera gel extract consisted of the inner leaf gel of hand-filleted Aloe vera leaves with the pulp removed. No further treatments were performed on this material prior to lyophilization.

The Aloe vera nondecolorized whole leaf extract was produced by grinding the whole leaves of Aloe vera plants and treating the slurry with cellulase (23 mg/L) to reduce viscosity and maximize yields. The Aloe vera nondecolorized whole leaf extract (referred to Aloe vera whole leaf extract in this technical report) contained the Aloe vera inner leaf gel and the Aloe vera latex, including the anthraquinones. Some Aloe vera latex anthraquinones are potent cathartic agents and induce laxation.

The Aloe vera decolorized whole leaf extract was produced in an identical manner as the Aloe vera whole leaf extract, with the exception that the slurry was further treated with activated carbon (1.0% wt/wt). Treatment of the whole leaf extract with activated charcoal removes the Aloe vera latex anthraquinone components from the extract.

Sterilization to maintain stability and kill endogenous bacteria in the Aloe vera test materials was achieved by gamma-ray irradiation using a cesium source to deliver the required dose range of 8 – 20 kGy (IBA/SteriGenics International, Shaumburg, IL).

For the 14-day studies, the Aloe vera gel extract lot numbers were 020318AG, 060308AG, 020810AG, and 022308AG; the Aloe vera whole leaf extract lot numbers were 020228ND, 060308ND, and 020928ND; and the Aloe vera decolorized whole leaf extract lot numbers were 020223AC, 060308AC, and 020916AC. For the 13-week studies, the Aloe vera whole leaf extract lot numbers were 042803ND, 032606ND, 081303ND, 082203ND, 090803ND, 093003ND, and 100203ND. For the 2-year studies, the Aloe vera whole leaf extract lot numbers were 041214ND, 040930ND, 041007ND, 041119ND, and 041210ND.

Once irradiated, the different lots of each of the different Aloe vera extracts were combined and blended, and new lot numbers were assigned. For the 14-day studies, Aloe vera gel extract was assigned lot numbers PA-02001 and PA-02002; Aloe vera whole leaf extract was assigned lot numbers WLN-02001 and WLN-2002; and Aloe vera decolorized whole leaf extract was assigned lot numbers WLD-02001 and WLD-2002. For the 13-week studies, Aloe vera whole leaf extract lots (042803ND, 032606ND, 081303ND, 082203ND, 090803ND, 093003ND, and 100203ND) were combined with WLN-02002, and the new lot was assigned lot WLN-03001. For the 2-year study, Aloe vera whole leaf extract was assigned lot numbers WLN-005001A, WLN-005001B, WLN-006001A, WLN-006001B, and WLN-006001C.

The irradiated lots of the Aloe vera extracts were stored at $\leq$ -20° C by the Diet Preparation staff (Bionetics, Inc., NCTR, Jefferson, AR), who also maintained custody, chemical usage log, and chain-of-custody documentation for each lot of each extract. The environmental temperatures of the facilities used to store the Aloe vera extracts were monitored with a Siemen's control system by the Division of Engineering, Operations, and Maintenance at NCTR.

The Chemistry Support Group in the Division of Biochemical Toxicology at NCTR was responsible for determining the homogeneity, stability, and chemical characterization of the Aloe vera extracts. For homogeneity analyses, the detection and quantification of the organic acid, malic acid, and aloin A, the principle anthraquinone in the Aloe

vera latex, were assessed in nine 50 mg samples randomly collected from the top, middle, and bottom of each lot of the irradiated bulk extracts used in the 14-day, 13-week, and 2-year studies.

For the 14-day studies, homogeneity testing showed that the contents of malic acid and aloin A were 116 – 212 mg/g and 1.1 – 1.4 mg/g, respectively, for Aloe vera gel; 188 – 197 mg/g and 14.1 – 15.9 mg/g, respectively, for the Aloe vera whole leaf extract; and 215 – 258 mg/g and 0.06 – 0.2 mg/g, respectively, for the Aloe vera decolorized whole leaf extract. In stability studies, the recovery of malic acid in 3% dosed water solutions stored at room temperature ranged from 87.8% - 97.1% of targeted amounts initially (day 0), and were 67% - 95.6% of targeted amounts at day 3. The stability of aloin A in 3% dosed water solutions decreased at room temperature. The percent recovery of targeted values for aloin A ranged from 90.3% to 188.0% at day 0, and decreased to 55.8% - 56.0% at day 3. The stability of malic acid and aloin A were greatly enhanced when the dosed water solutions were stored at 5°C, with little degradation detected even at 72 h.

For the 13-week studies, the results of homogeneity testing showed that the contents of malic acid and aloin A were 170.7 – 192.9 mg/g and 12.56 – 14.40 mg/g, respectively, for the Aloe vera whole leaf extract. The stability of malic acid and aloin A was examined in 0.5% and 3.0% dosed water solutions of the Aloe vera whole leaf extract for 96 h with storage at 2 - 8°C. The recovery of malic acid in dosed water solutions ranged from 92.9% – 97.2% of targeted amounts initially, and were 93.5% – 95.5% of targeted amounts at 96 h. The recoveries of aloin A in dosed water solutions showed significant degradation over the 96 h duration at 2 - 8°C. The percent of targeted values for aloin A ranged from 88.6% – 92.3%, initially, 83.1% – 87.3% at 48 h, and decreased to 77.6% – 83.0% at 96 h.

For the 2-year studies, the results of homogeneity testing on the bulk extracts showed that the content of malic acid in the five lots of Aloe vera whole leaf extract ranged from 186 ± 3 mg/g to 203 ± 3 mg/g (mean ± s.d.) and the content of aloin A ranged from 5.7 ± 0.2 mg/g to 7.2 ± 0.3 mg/g. Aloe emodin, an anthraquinone present in Aloe vera latex, was also assessed in nine randomly collected samples from one lot of the blended and irradiated Aloe vera whole leaf extract. The content of aloe emodin was 70.5 ± 4.5 µg/g. The stability of aloin A was assessed in 0.5, 1.0, 1.5, 2.0, and 3.0% (wt/wt) water solutions of the Aloe vera whole leaf extract lot #WLN-005001A for 96 h with storage at room temperature and storage at 2 - 8°C. The recovery of aloin A in 1.0% aqueous solution and

higher dose levels of the Aloe vera whole leaf extract stored at room temperature decreased to ≤ 80% of initial levels at 48 h. In Aloe vera whole leaf extract solutions stored at 2 - 8°C, the levels of aloin A at all dose levels were > 80% of initial levels at 72 h and were 95.4, 79.5, 79.8, 81.8, and 79.9% of initial levels for the 0.5, 1.0, 1.5, 2.0 and 3.0% dose levels, respectively, at 96 h.

Additional studies to determine the molecular weight and stability of the extract were performed at NCTR, and glycosyl linkage analysis was performed by the Complex Carbohydrate Research Center, University of Georgia (supported in part by NIH-funded Resource Center for Biomedical Complex Carbohydrates). The average molecular weight of the polysaccharide content of each lot of test article was determined by size exclusion chromatography with Rayleigh light-scattering detection.

For the 14-day studies, the results of molecular weight analyses showed that the polysaccharides in the Aloe vera gel extract had the highest average molecular weight at 3,000 ± 170 Kda (mean ± s.d, % CV=5.7) and the greatest extent of mannosyl residues, while the polysaccharides of the Aloe vera whole leaf and Aloe vera decolorized whole leaf extracts had average molecular weights of 91.9 ± 8.1 Kda (mean ± s.d, % CV=8.8) and 97.2± 16.3 Kda (mean ± s.d, % CV=16.8) and lower content of mannosyl residues.

In the 2-year studies, the average molecular weights of polysaccharides from each lot ranged from 52.1 ± 2.7 kDa to 78.3 ± 0.6 kDa (Table H7). Glycosyl composition analysis was performed on each lot of Aloe vera whole leaf extract by combined gas chromatography/mass spectrometry of the per-O-trimethylsilyl derivatives of the monosaccharide methyl glycosides produced from the samples by acidic methanolysis. Based on the results, the samples were similar in that 4 linked mannopyranose, 4 linked glucopyranose, and terminal glucopyranose were the most prominent glycosyl linkages (Table H6).

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The Bionetics, Inc. Diet Preparation support group prepared the dosed water formulations. For the 14-day range-finding and metabolism studies, aqueous 3% (wt/wt) master batch formulations of the lyophilized Aloe vera extracts were prepared on a daily basis (Monday - Sunday). Dissolution of the test articles in water was performed by gentle

mixing with a planetary mixer (Hobart, model KSM90) overnight in a walk-in cooler that was maintained at 4°C. For the 13-week and 2-year studies, aqueous 3% (wt/wt) master batch formulations of the lyophilized Aloe vera whole leaf extract were prepared three times weekly (Monday, Wednesday, and Friday) and twice weekly (Monday and Thursday), respectively. The dissolution of the test article in water was achieved by stirring for 2 h (Lightnin mixer, model EV1P25, Baldor Electric Co., Fort Smith, AR) in a walk-in cooler that was maintained at 4°C. Millipore 0.2 μm-filtered tap water served as the diluent for the dosed water formulations and as the control group treatment. For the 14-day studies, the final concentrations of the daily prepared dosed water formulations were 0, 0.5, 1.0, 1.5, 2.0, and 3.0% (wt/wt). For the 13-week studies and for the 2-year mouse study, the final concentrations of prepared dosed water formulations were 0, 1, 2, and 3% (wt/wt). For the 2-year rat study, the final concentrations of prepared dosed water formulations were 0, 0.5, 1.0, and 1.5% (wt/wt). Formulations were stored at 4°C until administered to animals.

The prepared dosed and control water formulations were dispensed into sterile 250 mL high-density polyethylene rodent water bottles using a calibrated pump and digital drive system (Masterflex 77300-40 L/S Pump and 77300-80 L/S Digital Modular Drive, Cole-Parmer Instrument Co., Vernon Hills, IL). For the 14-day studies, two different color-coded pull-ties were attached to the neck of the water bottles: one colored tie to indicate the specific Aloe vera extract used in the dosed water solution and the second colored tie to indicate the dose level of the solution. For the 13-week and 2-year studies, single colored pull-ties were used to indicate the concentration of the Aloe vera whole leaf extract in the solution. Color-coded labels of the same colors were affixed to cage cards to aide in the identification of treatment groups. Rubber bottle stoppers and stainless steel sipper tubes were inserted into the bottles, and bottles were encased in plastic wrap and stored at 4°C until used in the animal care facilities. Water bottles (used and unused) from the previous mix were removed at the time of delivery of freshly prepared dosed water bottles.

Samples of the control and each level of dosed water for each extract were collected from each mix and submitted to the Chemistry Support Group in the Division of Biochemical Toxicology at NCTR. Dose certifications for each dose level were conducted in a random order twice weekly for the 14-day studies and weekly for the 13-week and 2-year studies by HPLC analysis (Tables H2, H3, and H4, respectively). The detection and quantification of malic

acid and aloin A in the dosed water samples were compared to targeted concentrations of malic acid and aloin A obtained from the homogeneity test results on the different lots of the irradiated Aloe vera extracts. HPLC analyses were unable to detect malic acid or aloin A in control water samples.

The conditions of use and stability of the extracts in this study dictated that dosed waters were administered to the animals prior to completion of dose certification analyses. Therefore, while an acceptability range of ± 10% of target was desirable, the goal of the dose certification was to enable calculation of the dose being administered to an animal at a specific time point.

For the 14-day range-finding study, drinking water solutions of 0.5, 1.0, 1.5, 2.0, and 3.0% Aloe vera gel had malic acid contents of 1060, 2120, 3180, 4240, and 6360 μg/g water, respectively, and aloin A contents of 5.6, 11.1, 16.7, 22.2, and 33.3 μg/g water, respectively. Drinking water solutions of 0.5, 1.0, 1.5, 2.0, and 3.0% Aloe vera decolorized whole leaf had malic acid contents of 1240, 2480, 3720, 4960, and 7440 μg/g water, respectively, and aloin A contents of 0.8, 1.5, 2.2, 3.0, and 4.5 μg/g water, respectively. Drinking water solutions of 0.5, 1.0, 1.5, 2.0, and 3.0% of Aloe vera whole leaf extract had malic acid contents of 970, 1940, 2910, 3880, and 5820 μg/g water, respectively, and aloin A contents of 70, 141, 212, 282, and 422 μg/g water, respectively. The mean percentages of target values and coefficients of variation (CV %) for malic acid in dosed waters were 83.2 ± 9.1%, 87.3 ± 5.2%, and 94.3 ± 5.2% for the Aloe vera gel, Aloe vera decolorized whole leaf, and Aloe vera whole leaf, respectively. The mean percentages of target values and coefficients of variation (CV %) for aloin A in dosed waters were 102.1 ± 9.0%, 91.2 ± 8.0%, and 92.6 ± 5.9% for the extracts of Aloe vera gel, Aloe vera decolorized whole leaf, and Aloe vera whole leaf, respectively. For the 14-day metabolism study, mean percentages of target values for malic acid ranged from 84.0% for Aloe vera gel to 95.9% for Aloe vera whole leaf, and those for aloin A ranged from 88.2% for Aloe vera whole leaf to 99.1% for Aloe vera gel.

For the 13-week subchronic and metabolism studies, drinking water solutions of 1.0, 2.0, and 3.0% Aloe vera whole leaf extract had malic acid contents of 1830, 3660, and 5490 μg/g water, respectively, and aloin A contents of 129,

258, and 387µg/g water, respectively. The mean percentages of target values and standard deviations for malic acid and aloin A in dosed waters were 95 ± 4% and 104 ± 6%, respectively.

For the 2-year studies, drinking water solutions of 0.5, 1.0, 1.5, 2.0 and 3.0 Aloe vera whole leaf extract had average malic acid contents of 975, 1945, 2920, 3640, and 5835 µg/g water, respectively, and average aloin A contents of approximately 32.3, 65.6, 98.3, 131.3, and 196.8 µg/g water, respectively. The mean percentages of targeted values and standard deviations for malic acid and aloin A in dosed waters were 95 ± 7% and 100 ± 12%, respectively (Table H4). In general, the dosed water formulations were within the desirable acceptability range, with an overall average of 95% for malic acid and 100% for aloin A. A formulation error on January 11, 2007 resulted in dose certification results of less than 50% of targeted values for this date (Table H4).

14-DAY STUDIES

The 14-day range-finding and metabolism studies were conducted to evaluate the cumulative toxic effects of repeated exposure to Aloe vera plant extracts (Aloe vera gel, Aloe vera whole leaf, and Aloe vera decolorized whole leaf) and to determine the appropriate exposure concentrations of the Aloe vera extracts to be used in 13-week subchronic studies. Drinking water was the selected route of administration because Aloe vera products are consumed in liquid form by the public.

Weanling male and female F344/N Nctr rats and B6C3F$_1$/Nctr (C57BL/6N x C3H/HeN MTV$^-$) mice were obtained from the NCTR breeding colony, Jefferson, AR. For the range-finding studies, rat body weights were 35.4 – 40.8 g for females and 31.1 – 38.9 g for males; and mouse body weights were 12.7 – 13.8 g for females and 14.5 – 15.3 g for males. Animal weights for the metabolism study were 30.4 – 54.6 g for female rats, 33.0 – 60.0 g for male rats, 8.9 – 12.1 g for female mice, and 9.5 – 12.8 g for male mice.

Groups of four male and four female F344/N rats and B6C3F_1 mice were administered the Aloe vera test articles at concentrations of 0, 0.5%, 1.0%, 1.5%, 2.0%, or 3% (wt/wt) in drinking water for a period of 14 days. Additional groups of four male and four female F344/N rats and B6C3F_1 mice received the same concentrations of the Aloe vera extracts for the same duration of exposure and were designated metabolism study animals. The control and dosed water formulations were prepared daily, and water bottles were issued to individual animal cages on a daily basis (7 days/wk). Water bottle weights at the time of issuance and removal and individual animal body weights were recorded daily, and clinical observations and cage feed consumptions were recorded weekly.

Male and female rats and mice designated for the metabolism studies were placed individually into metabolism cages on day 4 and again on day 11 of the studies for a 24-hour urine collection and determination of gastrointestinal transit measurement. The design of the metabolism cages effectively separated feces and urine into 50 mL polypropylene tubes that attached to the outside of the cage.

Starting on day 5 and again on day 12, timed urine collections were performed for all metabolism animals for urine visual and chemistry evaluations. During the urine collection, the collection tubes were emersed into ice-filled insulated containers to minimize evaporation and suppress bacterial growth. Animals had access to feed and water during urine collection periods.

The physical examination of the urine (color and appearance) was conducted on a mixed 24-h urine sample prior to centrifugation for urinalysis. Volume determinations by measurement, color and appearance were determined using acceptable terminology (color; yellow, straw, bloody, or amber, and appearance; clear, slightly cloudy, cloudy, or turbid). Urine chemistry was performed on a Cobas Mira Plus Analyzer (Roche Diagnostic Systems, Sommerville, NJ), with Roche Diagnostic Systems reagents (creatinine, Jaffe method; glucose, Hexokinase method) and Wako reagents (micro protein, pyrogallol red method). Urines were centrifuged at 1000 g for 10 min before analysis. The instrument was calibrated daily with urine based standards and 2 levels of assayed controls were included in daily analyses as internal controls. All instrumentation maintentance was performed in accordance with manufacturer receommendations.

Gastrointestinal transit times were determined by monitoring the excretion of carmine red in animal; feces. On day 6 and day 13 of the studies, feed hoppers of NIH-31 rodent chow pellets were replaced with NIH-31 rodent meal that contained the dye carmine red (50 mg/100 g meal). Fecal collection tubes were checked hourly for the appearance of carmine red in the feces, and the first appearance was recorded. Animals housed in metabolism units were returned to their home cages on day 7 and again on day 14.

Animals were provided dosed water until euthanized. At the end of the study, rats and mice were weighed individually, anesthetized with carbon dioxide, and blood was collected by cardiac puncture until exsanguination. Clinical chemistries and hematology evaluations were performed on all animals by Toxicologic Pathology Associates (NCTR, Jefferson, AR). Whole blood for complete blood counts was collected in EDTA and analysis was performed the same day. The samples for clinical chemistry were allowed to clot and then centrifuged. The serum was removed and held frozen at -60° C until analyzed.

Complete blood counts, including leukocyte counts, erythrocyte counts, hemoglobin concentration, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration; and platelet counts were determined on a Cobas Minos Vet analyzer (Roche Diagnostic Systems, Somerville, NJ). Maintenance and calibration was performed in accordance with manufacturer recommendations. Three levels of assayed controls were included in daily analyses as internal controls. Clinical chemistry analyses were conducted between September 16, 2002 and February 21, 2003 on a Cobras Mira Plus analyzer (Roche Diagnostic Systems) with Roche Diagnostic reagents. The instrument was calibrated daily and 2 levels of assayed control were included in daily analyses as internal controls. All instrumentation maintentance was performed in accordance with manufacturer receommendations.

A complete necropsy was performed on all rats and mice. Organs including the brain, heart, liver, thymus, lungs, right kidney, spleen, and right testis were weighed. All gross lesions observed during necropsy were recorded on the individual animal necropsy record. The thyroid and parathyroid glands in all high dose and corresponding control

animals, the liver, thymus, lung, and kidney from the control and high dose animals exposed to the Aloe vera whole leaf extract, and all gross lesions were examined by histopathology.

13-WEEK STUDIES

The 13-week subchronic and metabolism studies were conducted to evaluate the cumulative toxic effects of repeated exposure to the Aloe vera whole leaf extract and to determine the appropriate exposure concentrations of the extract to use in 2-year bioassays. Drinking water was the selected route of administration because Aloe vera products are consumed in liquid form by the public.

Weanling male and female F344/N Nctr rats and B6C3F$_1$/Nctr (C57BL/6N x C3H/HeN MTV$^-$) mice were obtained from the NCTR breeding colony, Jefferson, AR. For the subchronic studies, body weights were 61.2 – 65.7 g in female rats and 64.4 – 71.5 g among male rats; and the body weights of mice were 11.0 – 11.3 g among females and 10.9 – 11.3 g among males. For the metabolism studies, body weights of rats were 38.2 – 38.6 g in females and 38.9 – 39.7g among males; and in mice, the body weights were 10.4 – 10.5 g among females and 11.2 – 11.4 g among males.

Animals were housed in standard polycarbonate rodent cages with hardwood chip bedding. Same sex rats were housed two per cage, and same sex mice were housed four per cage. Feed and water were available *ad libitum*. All animals were observed twice daily for well being. Baseline water and feed consumption data and individual animal body weights were collected daily for 1 week prior to start of dosing. Animals that demonstrated a loss in body weight or animal cages that showed low feed or fluid consumption during the baseline period were not used.

Groups of 12 male and 12 female F344/N rats and B6C3F$_1$ mice were administered drinking water daily that contained the Aloe vera whole leaf extract at concentrations of 0, 1.0%, 2.0%, or 3% (wt/wt) in Millipore-filtered (0.2 μm) tap water for a period of 13-weeks (92 days). Additional groups of 12 male and 12 female B6C3F$_1$ mice and F344/N rats received either 0 or 3.0% (mice) or 0 or 2.0% (rats) of the Aloe vera whole leaf extract for the same duration of exposure and were designated metabolism study animals for mechanistic evaluations.

The control and dosed water formulations were prepared 3 times weekly, and fresh water bottles were issued to animal cages daily (7 days/wk). Water bottle weights were recorded at the time of issuance and removal. Individual body weights and cage feed consumption were recorded weekly during the study, and clinical observations and cage feed consumptions were recorded weekly.

Male and female rats and mice designated for the metabolism studies were placed individually into metabolism cages on days 28, 56, and 84 for a 24-hour urine collection and determination of gastrointestinal transit measurement. Starting on days 29, 57, and 85, timed urine collections were performed for all metabolism animals for visual and chemistry evaluations. During the 24 h urine collection, the collection tubes were emersed into ice-filled insulated containers to minimize evaporation and suppress bacterial growth. Animals had access to feed and water during urine collection periods. Urine physical and chemical analyses were performed by Toxicologic Pathologists Associtates (Jefferson, AR) and were conducted on freshly collected samples.

The physical examination of the urine (color and appearance) was conducted on a mixed 24-h urine sample prior to centrifugation for urinalysis. Volume determinations by measurement, color and appearance were determined using acceptable terminology (color; yellow, straw, bloody, or amber, and appearance; clear, slightly cloudy, cloudy, or turbid). Urine chemistry was performed on a Cobas Mira Plus Analyzer (Roche Diagnostic Systems, Sommerville, NJ), with Roche Diagnostic Systems reagents (creatinine, Jaffe method; glucose, Hexokinase method) and Wako reagents (micro protein, pyrogallol red method). Urines were centrifuged at 1000 g for 10 min before analysis. The instrument was calibrated daily with urine based standards and 2 levels of assayed controls were included in daily analyses as internal controls. All instrumentation maintentance was performed in accordance with manufacturer recommendations.

Gastrointestinal transit times were determined by monitoring the excretion of carmine red in animal feces on days 30, 58, and 86 of the studies. Feed hoppers of NIH-31 rodent chow meal were replaced with NIH-31 rodent meal that contained the dye carmine red (50 mg/100 g meal) and metabolism units were checked hourly for the

appearance of carmine red in the feces and the first appearance was recorded. Animals housed in metabolism units were returned to the home cage on days 31, 59, and 87. Animals were removed from the study on day 92.

Animals were provided dosed water until euthanized. Rats and mice designated for the subchronic studies were weighed individually and euthanized by carbon dioxide asphyxiation. Rats and mice designated for the metabolism studies were weighed individually, anesthetized with carbon dioxide, and blood was collected by cardiac puncture until exsanguination. Clinical chemistries and hematology evaluations were performed on metabolism study animals by Toxicologic Pathology Associates (NCTR, Jefferson, AR). Whole blood for complete blood counts was collected in EDTA and analysis was performed the same day. The samples for clinical chemistry were allowed to clot and then centrifuged. The serum was removed and held frozen at -60° C until analyzed.

Complete blood counts, including leukocyte counts, leukocyte differential, erythrocyte counts; hemoglobin concentration, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration; and platelet counts were determined on a Cobas Minos Vet analyzer (Roche Diagnostic Systems, Somerville, NJ). Maintenance and calibration was performed in accordance with manufacturer recommendations. Clinical chemistry analyses were conducted between February 2, 2004 and April 28, 2004 on a Cobras Mira Plus analyzer (Roche Diagnostic Systems) with Roche Diagnostic reagents. The instrument was calibrated daily and 2 levels of assayed controls were included in daily analyses as internal controls. All instrumentation maintentance was performed in accordance with manufacturer receommendations.

A complete necropsy was performed on all animals, and gross observations were recorded on the IANR. Organs including the brain, heart, liver, thymus, lungs, right kidney, spleen, and right testis were weighed. All gross lesions and the spleen, kidneys, liver, colon, and cecum in all animals were examined by histopathology. Cecum and colon tissues from rats designated metabolism animals were perfused with physiologic saline, and the cecum and sections of the ascending, transverse, and descending colon were examined by histopathology. The remainder of the colon and cecum tissues from the metabolism studies were placed in liquid nitrogen and stored at -80^{o} C.

2-YEAR STUDIES

Study Design

Groups of 48 male and 48 female F344/N rats were administered the Aloe vera whole leaf extract at concentrations of 0, 0.5%, 1.0%, or 1.5% (wt/wt) in drinking water for a period of 104 weeks, with no recovery period. Groups of 48 male and 48 female B6C3F_1 mice were exposed to the Aloe vera whole leaf extract in the drinking water at concentrations of 0, 1.0%, 2.0% or 3.0% in drinking water for a period of 104 weeks, with no recovery period. Animals were housed in standard polycarbonate rodent cages with hardwood chip bedding. Same sex rats were housed two per cage, and same sex mice were housed four per cage. In a few instances, aggressive male mice were separately housed from non-aggressive cage companions. Feed and water were available *ad libitum*. Baseline water and feed consumption data and individual animal body weights were collected daily for 1 week prior to start of dosing. Animals that demonstrated a loss in body weight or animals in cages that showed low feed or fluid consumptions during the baseline period were replaced with healthier animals.

The control and dosed water formulations were prepared twice weekly and fresh water bottles were issued to individual animal cages on Monday, Wednesday, Friday, and Sunday of each week of the study.

Water bottle weights at the time of issuance to and removal from the animal cage were recorded. Individual body weights of rats and mice were recorded initially, weekly throughout the study, and at the end of the study. Cage feed consumption and clinical observations were recorded weekly during the study.

At the end of the study, rats and mice were weighed individually and fasted overnight. Animals were provided dosed water until euthanized by carbon dioxide asphyxiation. A complete necropsy and microscopic examination were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, except the eyes and testes were fixed in Davidson's fixative, processed and trimmed, embedded in infiltrating media (Formula R®), sectioned at

approximately 5 microns, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (i.e. adrenal gland, kidney, ovary) samples from each organ were examined.

Source and Specification of Animals

Weanling male and female F344/N rats and B6C3F$_1$ mice were obtained from the NCTR breeding colony, Jefferson, AR. For the 2-year studies, rats and mice were allocated separately and randomly assigned to treatment groups on a weight ranked basis at 5 weeks of age. The rats and mice were loaded over five allocations each. The body weights of rats ranged from 55.1 – 65.2 g among females and from 54.3 – 68.8 g among males. Within the same allocation, the maximum difference in the body weights of rats was less than 3.7 g in females and less than 2.1 g in males. Body weights ranged from 16.4 – 19.0 g in female mice and from 18.6 – 22.8 g among male mice. Within the same allocation, the maximum difference in the body weights of rats was less than 3.7 g in females and less than 2.1 g in males. The first load of rats and mice went on dose on April 25, 2005 and April 27, 2005, respectively, and the last rats and mice were euthanized on May 22, 2007 and May 23, 2007, respectively.

Once allocated to the study, mice were identified initially by ear clip; rats were identified by an 11-digit unique animal identification number, the last four digits of which were tattooed onto the animal tail (AIMS, Inc., Bud Lake, NJ). At 6-weeks of age, mice were also identified by an 11-digit unique animal identification number, the last four digits of which were tattooed onto the animal tail (AIMS, Inc.). The four digits of the tail tattoo corresponded to the animal cage number and the alpha-numeric character of the ear clip; ear clips of both, left, none, and right corresponded to the last tail tattoo digit of 1, 2, 3, and 4, respectively. An exception was made for aggressive male mice, where the tail tattoo numbers corresponded to their cage of origin and not their home cage. Rats and mice were between 6 and 7 weeks of age at the start of the dosed water treatments.

Animal Maintenance

All animal experimental procedures were performed in accordance with an animal study protocol approved by the National Center for Toxicological Research's Institutional Animal Care and Use Committee.

The rats and mice were housed in animal rooms in separate buildings. Animal cages were changed two times each week, and animal cage racks were changed and rotated every third week throughout the conduct of the studies. A mechanical problem with the air handling unit resulted in mice on the 2-year study undergoing an unplanned transfer to a new room within the same building on February 6, 2006. The transfer was deemed necessary to ensure that environmental conditions within the animal area were maintained and was not considered an action that would affect the outcome of the study. The environment of the animal rooms was monitored by a Siemens air handler computer system with controls set to maintain a temperature of 23 ± 3° C, a relative humidity of 50 ± 20%, and at least 10 air changes per hour. A 12-hour light cycle was maintained, with the dark cycle beginning no earlier than 6:00 pm CDT. Rats and mice on the 2-year studies were fed autoclaved NIH-31 rodent chow pellets (Purina Mills, Richmond, NJ), and feed and dosed water formulations were provided *ad libitum*. Microbiological surveillance of the drinking water, feed, cage waste, and room environments was conducted on a routine basis.

The NCTR Multi-Generation Support System (MGSS), an operator-prompted database system, was used to monitor the activities conducted by animal care technicians with the mice and rats in the animal rooms. Baseline animal feed and water consumption and individual animal body weights were collected daily. Once on dose, weekly body weights were recorded on individual rats and mice. Cage feed consumption was measured weekly, and the issuance and removal of water bottles were recorded for individual cages on Monday, Wednesday, Friday, and Sunday of each week. Sentinel animals were selected randomly for serological screening of viral and mycoplasma evaluations and gross observations at 6, 13, 19, and 25 months of the study for mice and at 6, 12, 18, and 25 months of the study for rats (Appendix L).

Clinical Examinations and Pathology

Visual inspection of cages for animal well-being was conducted twice daily, and clinical observations were recorded on individual rats and mice weekly.

At necropsy, all organs and tissues were examined for grossly visible lesions, and gross findings were recorded in the automated Gross Pathology System. Cross sections of the transverse and descending colon were flash frozen

and stored at -70°C; with the remaining colon tissue and liver preserved in 10% neutral buffered formalin for 48 h. All protocol-designated tissues were removed and preserved in 10% neutral buffered formalin, with the exception of the eyes and testes, which were placed in Davidson's fixative. All protocol-designated tissues, including the colon, were trimmed, processed, embedded in infiltrating media (Formula R®), sectioned at approximately 5 microns, and stained with hematoxylin and eosin for microscopic examination. When applicable, nonneoplastic lesions were graded for severity.

At the request of the study pathologist, an amendment to the pathology protocol on September 27, 2006, changed the processing procedures for the intestines of rats. After September 27, 2006, the entire intestinal tract was placed in physiological saline and the cecum and colon were flushed with physiological saline. The cecum was opened along its greater curvature, the contents removed, and the mucosa examined for gross lesions. The cecal-colic junction was opened, examined grossly, and a section preserved in 10% neutral buffered formalin and processed for histopathological evaluation. In the event of the presence of masses or nodules in the large intestine, the five largest nodules or masses were documented as gross lesions, and any nodule or mass greater than 5 mm in diameter was bisected into equal halves, with one half preserved in 10% neutral buffered formalin and the other half flash frozen and stored for special studies. When possible, a microscopic finding was recorded with the corresponding gross observation, and a primary cause of death, along with any contributing cause a death, was assigned for animals removed early from the study.

Microscopic evaluations were completed by the study pathologists, and the pathology data were entered into the Toxicology Data Management System. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory, Experimental Pathology Laboratories, Inc. A quality assessment pathologist re-examined all slides from all tumors and all potential target organs, which included the ileo-cecal-colic junction (referred to as the proximal colon of rats and mice), the large intestine (the cecum, the ascending, transverse and descending colon, and rectum of rats and mice), the small intestine (ileum, jejunum, and duodenum of mice and rats), the stomach (forestomach and glandular stomach of mice and rats), the pituitary gland of rats and female mice, and the thyroid gland and mandibular and mesenteric lymph nodes of male rats.

The quality assessment report and the reviewed slides were submitted to the National Toxicology Program Pathology Working Group (PWG) chairperson, who reviewed selected tissues and addressed any inconsistencies in the diagnosis made by the study and quality assessment pathologists. Representative histopathology slides of lesions found related to the administration of the Aloe vera whole leaf extract treatment, examples of diagnosis disagreements between the study and quality assessment pathologists, and lesions of interest were presented by the chairperson to the PWG for review. The PWG examined the tissues without knowledge of dose group or previously rendered diagnosis. The final diagnoses for reviewed lesions represent a consensus between the study pathologists, the quality assurance pathologist, and the PWG.

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water Studies of Aloe vera Extracts

14-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
U.S. FDA National Center for Toxicological Research (NCTR, Jefferson, AR)	U.S. FDA National Center for Toxicological Research (NCTR, Jefferson, AR)	U.S. FDA National Center for Toxicological Research (NCTR, Jefferson, AR)
Strain and Species		
Rats: F344/N Nctr Mice: B6C3F$_1$/Nctr (C57BL/6N x C3H/HeN MTV$^-$)	Rats: F344/N Nctr Mice: B6C3F$_1$/Nctr (C57BL/6N x C3H/HeN MTV$^-$)	Rats: F344/N Nctr Mice: B6C3F$_1$/Nctr (C57BL/6N x C3H/HeN MTV$^-$)
Animal Source		
NCTR breeding colony	NCTR breeding colony	NCTR breeding colony
Allocation Dates		
Range-finding Rats: August 22, 2002 Mice: August 28, 2002	Subchronic Rats: November 14, 2003 Mice: November 6, 2003	Rats: April 15, 22, & 29, 2005 and May 5 & 12, 2005 Mice: April 19 & 26, 2005 and May 3, 10, & 17, 2005
Metabolism Rats: November 19 & 22, 2002; December 9 & 23, 2002; and June 6, 2003 Mice: May 22 & 23 and June 6, 2003	Metabolism Rats: December 23, 2003 Mice: November 6, 2003	
Average Age When Studies Began		
7 weeks	6 to 7 weeks	6 to 7 weeks
Test Material		
Aloe vera gel, whole leaf, and decolorized whole leaf extracts	Aloe vera whole leaf extract	Aloe vera whole leaf extract
Date of First Exposure		
Range-finding Rats: September 2, 2002 Mice: September 9, 2002	Subchronic Rats: November 23, 2003 Mice: November 16, 2003	Rats: April 25, 2005 and May 2, 9, 16, & 23, 2005 Mice: April 27, 2005 and May 4, 11, 18, & 25, 2005
Metabolism Rats: October 31, 2002 Mice: June 12, 2003	Metabolism Rats: January 4, 2004 Mice: November 16, 2003	
Duration of Exposure		
14 days	91 to 92 days	104 weeks
Date of Last Exposure		
Range-finding Rats and Mice: September 26, 2002	Subchronic Rats: February 24, 2004 Mice: February 17, 2004	Rats: May 22, 2007 Mice: May 23, 2007
Metabolism Rats: January 23, 2003 Mice: July 10, 2003	Metabolism Rats: April 5, 2004 Mice: February 18, 2004	
Necropsy Dates		
Range-finding Rats: September 16 – 19, 2002 Mice: September 23 – 26, 2002	Rats Metabolism: April 6, 2004 Subchronic: February 24 – 25, 2004	Rats: April 24, May 1, 8, 15, and 22, 2007 Mice: April 25, May 2, 9, 16, and 23, 2007
Metabolism Rats: November 14 and December 19 & 23, 2002, and January 23, 2003 Mice: June 26 & 30, and July 10, 2003	Mice Metabolism: February 19, 2004 Subchronic: February 17 – 18, 2004	

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water Studies of Aloe vera Extracts (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Average Age at Necropsy 9 weeks	19-20 weeks	110-111 weeks
Size of Study Groups 4 males and 4 females	12 males and 12 females	48 males and 48 females
Method of Distribution Animals were randomly assigned to treatment groups on a weight ranked basis.	Animals were randomly assigned to treatment groups on a weight ranked basis.	Animals were randomly assigned to treatment groups on a weight ranked basis.
Animals per Cage Rats: 2, same sex Mice: 4, same sex	Same as 14-day studies	Same as 14-day studies
Method of Animal Identification Ear clip and tail tattoo	Same as 14-day studies	Same as 14-day studies
Diet Autoclaved NIH 31 rodent chow pellets (Purina Mills, Richmond, NJ), available *ad libitum*	Same as 14-day studies	Same as 14-day studies
Water Millipore 2 μm-filtered tap water containing dose formulations was available *ad libitum*	Same as 14-day studies	Same as 14-day studies
Cages Polycarbonate cages (Lab Products, Inc., Seaford, DE and Allentown Caging and Equipment, Allentown, NJ), changed twice weekly (rats) or once weekly (mice)	Same as 14-day studies	Same as 14-day studies
Bedding Autoclaved hardwood chip bedding (Northeastern Products Corp., Caspian, MI), changed twice weekly (rats and female mice) or once weekly (male mice)	Same as 14-day studies	Same as 14-day studies
Cage Filters Spunbonded polyester (Lab Products, Inc., Seaford, DE and Allentown Caging and Equipment, Allentown, NJ), changed every 2 weeks	Same as 14-day studies	Same as 14-day studies
Racks Stainless steel (Research Equipment Co., Bryan, TX), changed every 3 weeks	Same as 14-day studies	Same as 14-day studies
Animal Room/Chamber Environment Temperature: 23° ± 3°C Relative humidity: 50 ± 20% Room fluorescent light: 12 hours/day Room air change: ≥ 10/hour	Same as 14-day studies	Same as 14-day studies
Exposure Concentrations Rats and Mice: 0.0, 0.5, 1.0, 1.5, 2.0, and 3.0% (wt/wt) in drinking water, available *ad libitum*	Rats and Mice: 0.0, 1.0, 2.0, and 3.0% (wt/wt) in drinking water, available *ad libitum*	Rats: 0.0, 0.5, 1.0, and 1.5% (wt/wt) in drinking water, available *ad libitum* Mice: 0.0, 1.0, 2.0, and 3.0% (wt/wt) in drinking water, available *ad libitum*

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water Studies of Aloe vera Extracts (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Type and Frequency of Observation		
Observed twice daily; animals were weighed daily; water consumption was measured daily; cage feed consumption was measured weekly; clinical observations were recorded weekly	Observed twice daily; animals were weighed weekly; water consumption was measured daily; cage feed consumption was measured weekly; clinical observations were recorded weekly	Observed twice daily; animals were weighed weekly; water consumption was measured 4 times per week; cage feed consumption was measured weekly; clinical observations were recorded weekly
Urinalysis		
Timed collection (24 h) of urine from metabolism groups on days 5 and 12 for analysis of: total volume, urine creatinine, micro protein, urine glucose, 24 h urine creatinine, 24 h micro protein, and 24 h urine glucose.	Timed collection (24 h) of urine from metabolism groups on days 29, 57, and 85 for analysis of: total volume, urine creatinine, micro protein, urine glucose, 24 h urine creatinine, 24 h micro protein, and 24 h urine glucose.	None.
Gastrointestinal Transit		
Hourly monitoring of feces from metabolism groups on days 6 and 13 for excretion of carmine red; first appearance recorded.	Hourly monitoring of feces from metabolism groups on days 30, 58, and 86 for excretion of carmine red; first appearance recorded.	None.
Method of Sacrifice		
Anesthetized with carbon dioxide, and blood collected by cardiac puncture until exsanguination	Carbon dioxide asphyxiation (subchronic animals) Anesthetized with carbon dioxide, and blood collected by cardiac puncture until exsanguination (metabolism animals)	Carbon dioxide asphyxiation
Necropsy		
Necropsies were performed on all study animals. Organs weighed were heart, right kidney, lungs/bronchi, spleen, right testis, and thymus.	Necropsies were performed on all study animals. Organs weighed were brain, heart, right kidney, lungs/bronchi, spleen, right testis, and thymus.	Necropsies were performed on all study animals.
Histopathology		
In addition to gross lesions and tissue masses, the following tissues were examined: all thyroid and parathyroid glands from the high dose and control groups.	Complete histopathology was performed on animals that were removed early from the study, control animals, and all animals in the highest dose group with at least 60% survival to termination and all animals in higher dose groups. In addition to gross lesions and tissue masses, the following tissues were examined: spleen, kidneys, liver, colon, and cecum.	Complete histopathology was performed for all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone (including marrow) femur, brain (cerebellum, cerebrum, brain stem), clitoral gland, epididymus, esphagus, eyes, gall bladder, harderian gland, heart and aorta, large intestine (including cecum, colon, rectum), small intestine (including duodenum, jejunum, ileum), kidney, liver, lungs/bronchi, lymph node (including mesenteric and mandibular), nose, ovary, pancreas, parathyroid gland, pituitary gland, prostate, salivary gland, seminal vesicles, skin, mammary, spleen, stomach (including forestomach and glandular), testes, thymus, thyroid gland, trachea, urinary bladder, and uterus.

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water Studies of Aloe vera Extracts (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Clinical Pathology		
Hematology: hemoglobin concentration, mean cell volume, mean cell hemoglobin concentration, and erythrocyte, leukocyte, and platelet counts.	*Hematology*: hemoglobin concentration, mean cell volume, mean cell hemoglobin concentration, and lymphocyte, neutrophil, monocyte, basophile, erythrocyte, and platelet counts.	None.
Clinical chemistry: cholesterol, triglycerides, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatine, albumin, protein, glucose, amylase, creatine kinase, calcium, and phosphorus.	*Clinical chemistry*: cholesterol, triglycerides, alanine aminotransferase, blood urea nitrogen, creatine, albumin, protein, glucose, amylase, creatine kinase, calcium, sodium, potassium, chloride, and phosphorus.	

Statistical Methods

Survival Analyses

Animal survival data was extracted from the Genesis database, and uncensored and censored observations were assigned to the rats and mice; uncensored animals were disposed as dead or moribund, while censored animals had terminal dispositions. Kaplan-Meier estimates of mean survival times were calculated for each species by sex and dose. A Cox proportional hazard model was used to test the effect of treatment relative to control.

Body Weight Analyses

A one-way repeated measures mixed model analysis of variance (ANOVA) was performed for each sex, with terms for the dose, week, and all interactions. Week was treated as the repeated measure. Data at 4 week intervals from week 0 through 104 were included in these comparisons. Within-group correlations were modeled using a heterogeneous first-order autoregressive correlation structure, which allowed for correlated differences in variability across time points. Dunnett's test was performed for comparisons of dosed groups to the control groups.

Water and Feed Consumption Analyses

Feed and water consumption for each cage and for each consumption period (weekly for feed; approximately every 2nd day for water) were calculated by subtracting the container weight at the end of the period from the container weight at the beginning of the period. The average food or water consumption per animal per day in 4 week intervals from week 0 through week 104 was calculated for each cage by dividing the total food or water consumed by the number of animal-days. The number of animal-days was calculated by summing the number of animals in each cage across all days within a given time period.

Pairwise comparisons of means were made using contrasts within one-way repeated measures mixed model ANOVA for each sex, with terms for dose, week, and all interactions. Week was treated as the repeated measure. Within-group correlations were modeled using a heterogeneous first-order autoregressive correlation structure, which allowed for correlated differences in variability across time points. Dunnett's test was performed for comparisons of dosed groups to the control groups.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables 4, 14, A1, A4, B1, B4, C1, C3, D1, and D3 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidence of neoplasms and nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. Tables A2 and B2 also give the age-adjusted neoplasm rate for each treatment group and each site-specific neoplasm. This adjusted rate (based on the Poly-3 method described in the next section) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, to animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidence

Histopathology data were extracted directly from Toxicology Data Management System into the Laboratory Data Acquisition System, and lesions with an incidence greater than 5% were summarized. A modified Poly-3 method (Bailer and Portier, 1988; Bieler and Williams, 1993) and the National Institutes of Environmental Health Sciences continuity-correction method were used to analyze age-adjusted incidences of neoplastic lesions. For nonneoplastic lesions, the modified Poly-3 method was used to analyze age-adjusted incidences and non-zero severity scores were computed.

Analysis of Continuous Variables

Body weight, organ weight, hematology, clinical chemistry, urinalysis, and gastrointestinal transit for the 14-day and 13-week studies were analyzed separately; however, data for the 14-day range-finding and metabolism studies were combined prior to analysis. A one-way repeated measures of analysis of variance was used to analyze body weight data by sex, with terms for the Aloe vera extracts, dose, and day. Organ weights, hematology, clinical chemistry, urinalysis, and gastrointestinal transit were analyzed with a general linear model procedure and one-way analysis of variance for each sex. Organ weights relative to necropsy body weights were calculated and used to obtain least squares means in the analyses. Contrasts were used to determine linear dose trend effects, and multiple comparison

procedures of Dunnett (1955) were performed. Mean least squares values were compared to published reference values for the species.

Historical Control Data

Although historical control data can be helpful in the overall assessment of neoplasm incidences, spontaneous neoplasms of the large intestine do not occur with any frequency in the NCTR historical control database for the F344/N rat or the B6C3F1 mouse.

Quality Assurance and Archival of Data

This study was conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Unit at the NCTR performed audits and inspections of the protocols, procedures, data, and reports throughout the course of the study. Separate audits for completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this technical report were conducted. Audit procedures and audit findings for the study are maintained by the Quality Assurance Unit at the NCTR. The audit findings were reviewed and assessed by the NCTR staff, and all comments were resolved or addressed either before or during the preparation of the technical report.

Raw data sheets from the study are archived by the Records Management Unit at the NCTR. Histopathology samples collected during the course of the study are stored in the archives of Toxicologic Pathology Associates at the NCTR. Backup computer data are maintained by the computer staff at the NCTR. All records and samples are stored in accordance with Food and Drug Administration Good Laboratory Practice Regulations.

RESULTS

RATS

14-Day STUDY

Aloe vera gel extract. All rats survived until the end of the study (Table 2). Mean body weights and body weight gains of Aloe vera gel extract treated male and female rats were similar to those of controls (Table 2). Feed consumption of male and female rats was similar to those of controls (Table I1). Water consumption by 1, 1.5, and 2% Aloe vera gel groups of female rats were significantly higher than controls (Table 2). Dose-related decreases in serum levels of cholesterol, triglycerides, and albumin were observed, and triglycerides were significantly lower than controls in 1.5% and 2.0% Aloe gel groups of female rats and in the 3.0% group of male and female rats (Table E1). The urine chemistry for male and female rats administered Aloe vera gel extract is listed in Table E3. Dose related increases in 24 h urine glucose levels were observed at week 1 in male rats administered the Aloe vera gel extract. Gastrointestinal transit time (Table G1) and organ weights (Table F1) of Aloe vera gel extract treated male and female rats were similar to those of controls. No treatment-related findings were observed.

Aloe vera decolorized whole leaf extract. All rats survived until the end of the study (Table 2). Mean body weight gains of 3% male rats were significantly higher than those of controls, and dose-related increases in body weight gains were observed (Table 2). Feed consumption of male and female rats was similar to those of controls (Table I1). Hematology values were similar to those of controls in male rats (Table E1). Female rats demonstrated dose-related decreases in the levels of blood urea nitrogen, alanine aminotrasferase, and creatinine, and significantly lower blood urea nitrogen and creatinine levels that those of controls were found for the 3% Aloe vera decolorized group of female rats. The urine chemistry for male and female rats administered Aloe vera decolorized whole leaf extract are listed in Table E3. Urine physical and chemistry values for treated male and female rats were similar to controls. Gastrointestinal transit times (Table G1) and organ weights (Table F1) of Aloe vera decolorized whole leaf extract groups were similar to those of controls. No treatment-related findings were observed.

Aloe vera whole leaf extract. All rats survived until the end of the study (Table 2). Significant dose-related decreases in mean body weights were observed in male and female rats administered the Aloe vera whole leaf extract in the drinking water. The mean body weights of 3% Aloe vera whole leaf-treated rats were significantly less than those of controls at dose day 7 in males and at dose day 14 in both male and female rats. Significant dose-related lower body weight gains were observed in both sexes of rats (Table 2). Final body weights relative to controls were 79% in male rats and 81% in female rats administered the 3.0% dose level of Aloe vera whole leaf extract. There was a significant dose-related decrease in feed consumption at the end of week 1 in male rats and at the end of week 2 in female rats that were administered the Aloe vera whole leaf extract (Table I1). In comparison to controls, male rats administered the 3.0% dose of Aloe vera whole leaf extract had significantly lower feed consumption at week 1 and 2 of the study compared to that of controls. Water consumption by female rats showed dose-related decreases and levels were significantly lower than those of controls in 3% female rats (Table 2). A significant two-fold increase in leukocyte counts occurred in male and female rats exposed to the Aloe vera whole leaf extract, suggesting a potential inflammatory response (Table E1). Erythrocyte counts and hematocrit values were significantly elevated above control levels in 3% male and female rats. Values for these parameters were within stated reference value ranges for the laboratory rat, and the significance of these findings were uncertain.

The mean gastrointestinal transit times of carmine red dye are shown for male and female rats administered Aloe vera extracts in Table G1. Male rats administered the 1.0, 1.5, and 2.0% dose concentrations of Aloe vera whole leaf extract had significantly faster transit times of carmine red dye at week 1 of dosing, and 1.0, 1.5, 2.0, and 3.0% dose levels had significantly faster transit times at week 2 of dosing. Aloe vera whole leaf concentrations of 0.5% and above decreased transit times at week 1 of dosing in female rats, but only the 3.0% level had a significantly faster transit time at week 2 of dosing. The urine chemistry for male and female rats administered Aloe vera whole leaf extract is listed in Table E3. In comparison tests with controls, urine volumes were significantly lower and creatinine levels significantly higher at week 1 and week 2 of dosing in Aloe vera whole leaf extract treated male and female rats. Total protein mg/24-h levels demonstrated dose-related increases at week 2 in males and females.

All protocol-specified tissues were examined grossly at necropsy, removed, and preserved in 10% neutral buffered formalin. Gross lesion descriptions were recorded on the Individual Animal Necropsy Records. Specified tissues were trimmed, processed, embedded, sectioned at approximately 5 μm, and stained with hematoxylin and eosin. No treatment-related findings were observed in liver, thymus, lung, and kidney from control and high dose animals exposed to the Aloe vera whole leaf extract. All protocol-specified tissues were examined grossly at necropsy, removed, and preserved in 10% neutral buffered formalin. Gross lesion descriptions were recorded on the Individual Animal Necropsy Records. Specified tissues were trimmed, processed, embedded, sectioned at approximately 5 μm, and stained with hematoxylin and eosin. No treatment-related findings were observed in liver, thymus, lung, and kidney from control and high dose animals exposed to the Aloe vera whole leaf extract. No further microscopic examination of lower dose groups was performed. The absence of treatment-related lesions in organs with increased absolute or relative organ weight suggested that the increase in organ weights was likely attributable to a reduction in body weight due to dehydration. Clinical findings observed in groups of male and female rats exposed to the 1% and higher concentrations of the Aloe vera whole leaf extract included thinness, hunched posture, discolored fur, and diarrhea.

Exposure Concentration Selection Rationale: All rats survived the 14-day study with no treatment-related gross or microscopic lesions. Aloe vera whole leaf extract was selected as the test article for further studies, since it contains all of the Aloe vera constituents. Based upon the activity of Aloe vera extracts in the 14-day study, doses selected for the subsequent 13-week subchronic study were 0, 1, 2, and 3% (wt/wt). In 13-week metabolism studies, a 2.0% (wt/wt) dose of the Aloe vera whole leaf extract was selected.

TABLE 2
Survival, Body Weights, and Water Consumption of Rats in the 14-Day Drinking Water Study of Aloe vera Extracts

Aloe vera Extract and Concentration (%)	Survival[a]	Mean Body Weight[b] (g)				Final Weight Relative to Controls (%)	Mean Water Consumption[c]		
		Day 0	Day 7	Day 14	Change		Week 0	Week 1	Week 2
Male									
Gel									
0	8/8	135.2 ± 5.5	160.1 ± 6.1	175.7 ± 5.8	40.5 ± 2.7		20.49 ± 0.69	18.79 ± 0.98	22.65 ± 1.09
0.5	8/8	134.8 ± 5.5	161.0 ± 6.1	175.7 ± 5.8	40.9 ± 2.7	100	20.85 ± 0.69	19.60 ± 0.98	23.89 ± 1.09
1	8/8	130.1 ± 5.5	156.3 ± 6.1	172.4 ± 5.8	42.3 ± 2.7	98	21.07 ± 0.69	21.67 ± 0.98	23.36 ± 1.09
1.5	8/8	135.3 ± 5.5	161.0 ± 6.1	177.0 ± 5.8	41.7 ± 2.7	101	21.42 ± 0.69	20.76 ± 0.98	24.43 ± 1.11
2	8/8	135.2 ± 5.5	161.3 ± 6.1	178.8 ± 5.8	43.6 ± 2.7	102	22.88 ± 0.69	20.77 ± 0.98	22.41 ± 1.09
3	8/8	133.3 ± 5.5	156.8 ± 6.1	175.2 ± 5.8	41.9 ± 2.7	100	21.14 ± 0.69	20.19 ± 0.98	22.61 ± 1.09
Decolorized Whole Leaf									
0	8/8	133.7 ± 6.9	160.5 ± 7.4	179.7 ± 7.2	46.1 ± 2.4		20.99 ± 0.64	18.10 ± 0.78	21.78 ± 1.06*
0.5	8/8	133.8 ± 6.9	161.6 ± 7.4	178.6 ± 7.2	44.8 ± 2.4	99	19.38 ± 0.64	20.09 ± 0.78	22.38 ± 1.06
1	8/8	130.6 ± 6.9	158.3 ± 7.4	173.2 ± 7.2	42.6 ± 2.4	96	19.54 ± 0.64	18.45 ± 0.78	21.45 ± 1.06
1.5	8/8	131.5 ± 6.9	158.4 ± 7.4	176.0 ± 7.2	44.5 ± 2.4	98	20.35 ± 0.64	18.43 ± 0.78	23.56 ± 1.06
2	8/8	135.0 ± 6.9	162.4 ± 7.4	181.1 ± 7.2	46.1 ± 2.4	101	20.59 ± 0.64	18.79 ± 0.78	25.46 ± 1.06
3	8/8	131.6 ± 6.9	159.2 ± 7.4	178.0 ± 7.2	46.4 ± 2.4	99	20.40 ± 0.64	18.92 ± 0.78	24.53 ± 1.06
Whole Leaf									
0	8/8	134.2 ± 6.0	161.2 ± 7.5*	176.1 ± 7.6*	41.9 ± 4.2*		20.57 ± 0.62	21.79 ± 1.37*	21.89 ± 1.15
0.5	8/8	131.8 ± 6.0	157.2 ± 7.5	173.3 ± 7.6	41.5 ± 4.2	98	19.66 ± 0.62	21.82 ± 1.37	23.29 ± 1.15
1	8/8	129.9 ± 6.0	152.3 ± 7.5	169.0 ± 7.6	39.2 ± 4.2	96	20.09 ± 0.62	20.72 ± 1.37	23.41 ± 1.15
1.5	8/8	129.5 ± 6.0	148.4 ± 7.5	165.3 ± 7.6	35.8 ± 4.2	94	20.74 ± 0.62	21.10 ± 1.37	24.00 ± 1.15
2	8/8	131.4 ± 6.0	145.2 ± 7.5	159.4 ± 7.6	28.1 ± 4.2	91	21.04 ± 0.63	19.39 ± 1.37	22.74 ± 1.15
3	8/8	125.7 ± 6.0	131.0 ± 7.5*	139.0 ± 7.6*	13.4 ± 4.2*	79	20.60 ± 0.62	17.50± 1.37	21.36 ± 1.15

TABLE 2
Survival, Body Weights, and Water Consumption of Rats in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

Aloe vera Extract and Concentration (%)	Survival[a]	Mean Body Weight[b] (g)				Final Weight Relative to Controls (%)	Mean Water Consumption[c]		
		Day 0	Day 7	Day 14	Change		Week 0	Week 1	Week 2
Female									
Gel									
0	8/8	102.0 ± 5.0	115.1 ± 4.6	119.7 ± 4.2	17.7 ± 1.5		18.46 ± 0.61	16.31 ± 0.97	17.69 ± 0.85
0.5	8/8	112.3 ± 5.0	125.0 ± 4.6	129.1 ± 4.2	16.8 ± 1.5	108	18.63 ± 0.61	17.00 ± 0.97	20.57 ± 0.85
1	8/8	113.7 ± 5.0	128.3 ± 4.6	131.2 ± 4.2	17.5 ± 1.5	110	19.79 ± 0.61	19.62 ± 0.97	22.10 ± 0.85*
1.5	8/8	108.9 ± 5.0	122.0 ± 4.6	125.8 ± 4.2	16.9 ± 1.5	105	18.39 ± 0.61	17.54 ± 0.97	20.78 ± 0.85*
2	8/8	112.8 ± 5.0	125.4 ± 4.6	129.8 ± 4.2	17.0 ± 1.5	108	19.01 ± 0.61	21.01 ± 0.97*	20.85 ± 0.85*
3	8/8	111.1 ± 5.0	123.0 ± 4.6	131.3 ± 4.2	20.1 ± 1.5	110	18.35 ± 0.62	16.51 ± 1.01	18.92 ± 0.85
Decolorized Whole Leaf									
0	8/8	109.9 ± 4.4	125.5 ± 4.3	126.5 ± 3.6	16.6 ± 1.6*		18.71 ± 0.56	18.52 ± 0.63	21.01 ± 0.95*
0.5	8/8	110.9 ± 4.4	124.9 ± 4.3	128.0 ± 3.6	17.1 ± 1.6	101	18.83 ± 0.56	16.83 ± 0.63	19.61 ± 0.95
1	8/8	112.8 ± 4.4	126.1 ± 4.3	129.5 ± 3.6	16.7 ± 1.6	102	19.11 ± 0.56	16.66 ± 0.63	20.41 ± 0.95
1.5	8/8	115.5 ± 4.4	129.3 ± 4.3	131.2 ± 3.6	15.7 ± 1.6	104	17.27 ± 0.56	15.62 ± 0.63	21.80 ± 0.95
2	8/8	111.8 ± 4.4	126.4 ± 4.3	129.7 ± 3.6	17.9 ± 1.6	103	19.48 ± 0.56	16.67 ± 0.63	21.93 ± 0.95
3	8/8	107.8 ± 4.4	123.1 ± 4.3	130.6 ± 3.6	22.7 ± 1.6*	103	18.52 ± 0.56	16.80 ± 0.63	23.76 ± 0.95
Whole Leaf									
0	8/8	106.7 ± 3.1	119.4 ± 4.0*	121.4 ± 4.5*	14.7 ± 3.8*		20.07 ± 0.65	17.46 ± 0.84*	17.50 ± 0.85*
0.5	8/8	106.8 ± 3.1	119.7 ± 4.0	123.1 ± 4.5	16.3 ± 3.8	101	19.14 ± 0.65	17.35± 0.84	18.36 ± 0.85
1	8/8	108.4 ± 3.1	120.6 ± 4.0	123.6 ± 4.5	15.1 ± 3.8	102	19.38 ± 0.65	16.43 ± 0.84	16.89 ± 0.85
1.5	8/8	105.4 ± 3.1	114.7 ± 4.0	117.1 ± 4.5	11.6 ± 3.8	96	18.11 ± 0.65	15.33 ± 0.84	16.52 ± 0.85
2	8/8	103.5 ± 3.1	106.5 ± 4.0	109.3 ± 4.5	5.9 ± 3.8	90	20.24 ± 0.65	14.03 ± 0.84*	15.30 ± 0.85
3	8/8	109.0 ± 3.1	105.2 ± 4.0	98.0 ± 4.5*	-11.1 ± 3.8*	81	20.80 ± 0.65	13.20 ± 0.87*	12.13 ± 0.85*

[a] Number of animals surviving at 14 days/number initially in group.
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.
* Signifies values that are significantly different ($P \leq 0.05$) from control group by Dunnett's test and in the control group, significant linear dose trend ($P \leq 0.05$) effects based on contrast comparisons.

13-WEEK STUDY

Early removal of rats from the study due to death or morbidity only occurred in the 2.0 and 3.0% Aloe vera whole leaf extract dose groups (Table 3). One male rat in the 1.0% Aloe vera whole leaf extract dose group was discarded after 74 days on the experiment due to technician error, when the rat was erroneously administered the incorrect dose for 24 – 48 h. Two male and 4 female 2.0% rats and 5 male and 8 female 3.0% rats were removed due to death or morbidity before the end of the study.

Initial (Day 0) body weights did not differ from controls at the start of the dosing period. Significant dose-related decreases were observed in male and female rats administered the Aloe vera whole leaf extract beginning at week-4 of the study and continuing until study termination. Final mean body weights and mean body weight gains of these animals showed significant dose-related decreases. Final mean body weights relative to controls were 71.84% and 77.37%, respectively, for 3.0% Aloe vera whole leaf male and female groups (Table 3). Similar findings occurred in rats on the metabolism study. Final body weights relative to controls were 67.17% and 85.71%, respectively, for the 2.0% Aloe vera whole leaf male and female groups (Table 3).

Feed consumption by male and female rats administered the Aloe vera whole leaf extract was significantly less than that of the controls at week 4 of the study, and significant dose-related decreases occurred at week 13 of dosing in male rats (Table I2). Polydipsia was prevalent in male and female rats administered the Aloe vera whole leaf extract. Water consumption by rats administered the Aloe vera whole leaf extract were significantly higher than controls, with significant dose-related increases observed at days 60 and 90 in male rats and at day 90 in female rats (Table 3). Mean daily water consumptions of male rats in the 3.0% Aloe vera whole leaf groups were 40 and 41 grams compared to 23 and 19 grams for male controls at days 60 and 90, respectively. Mean daily water consumption for 3.0% Aloe vera whole leaf female rats was 26 g on day 60 and 25 g on day 90, compared to 20 g and 17 g, respectively, for controls. The mean intake of water by 3.0% male and female rats were equivalent to 4.0 and 3.2 g Aloe vera whole leaf per kg body weight, respectively, and equated to 732 and 585 mg of malic acid/kg body weight/day and 51.6 and 41.3 mg of aloin A/kg body weight/day, respectively.

Clinical findings observed in groups of male and female rats administered the Aloe vera whole leaf extract included thinness, hunched posture, discolored urine, and diarrhea.

TABLE 3
Survival, Body Weights, and Water Consumption of Rats in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract

Concentration (%)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Mean Water Consumption[c]			
		Dose Day 0	Day 92	Change		Day 0	Day 30	Day 60	Day 90
Subchronic Study									
Male									
0	12/12	122.1 ± 4.7	339.5 ± 6.7*	217.5 ± 7.4*		19.1 ± 3.0	20.9 ± 3.3	23.0 ± 2.9*	23.6 ± 2.6*
1	11/12	130.1 ± 4.7	304.3 ± 7.0*	172.6 ± 7.7*	89.6	17.9 ± 3.0	29.7 ± 3.3	40.7 ± 2.9*	41.0 ± 2.6*
2	10/12	122.1 ± 4.7	264.4 ± 7.3*	144.5 ± 8.1*	77.9	23.4 ± 3.0	32.7 ± 3.3	41.2 ± 2.9*	45.7 ± 2.9*
3	7/12	120.7 ± 4.7	243.9 ± 8.8*	127.2 ± 9.7*	71.8	16.7 ± 3.0	23.3 ± 3.3	40.0 ± 3.1*	46.8 ± 2.9*
Female									
0	12/12	94.3 ± 5.4	188.4 ± 3.5*	94.1 ± 5.9*		13.9 ± 2.2	20.4 ± 2.2	19.7 ± 3.3	19.6 ± 2.8
1	12/12	105.7 ± 5.4	183.1 ± 3.5	77.4 ± 5.9	97.2	21.5 ± 2.2	21.5 ± 2.2	25.7 ± 3.3	19.9 ± 2.8
2	8/12	103.0 ± 5.4	156.7 ± 4.3*	55.7 ± 7.2*	83.2	15.0 ± 2.2	19.6 ± 2.2	43.0 ± 3.3*	31.2 ± 2.8*
3	4/12	96.3 ± 5.4	145.8 ± 6.1*	43.8 ± 10.2*	77.4	14.3 ± 2.2	19.0 ± 2.7	25.9 ± 4.7	26.1 ± 3.9
Metabolism Study									
Male									
0	12/12	114.9 ± 2.0	316.6 ± 6.4	201.7 ± 5.4	93.3	20.5 ± 0.6	7.9 ± 2.1	30.2 ± 1.9	23.8 ± 1.9
2	12/12	114.5 ± 2.0	228.1 ± 6.4	113.6 ± 5.4	67.2	20.4 ± 0.6	22.4 ± 2.1*	42.8 ± 1.9*	47.5 ± 1.9*
Female									
0	12/12	99.6 ± 1.2	189.3 ± 3.3	89.7 ± 3.0	100.5	17.3 ± 0.3	12.3 ± 1.6	19.3 ± 1.8	18.8 ± 1.0
2	9/12	101.3 ± 1.2	161.5 ± 3.9	59.2 ± 3.5	85.7	17.3 ± 0.3	13.9 ± 1.6	30.9 ± 2.0*	24.5 ± 1.1*

[a] Number of animal surviving until study termination/number of animals initially in group.
[b] Weights and weight changes are given as mean ± standard error of the mean.
[c] Water consumption is given as mean ± standard error of the mean and is expressed as grams per animal per day.
* Signifies values that are significantly different ($P \leq 0.05$) from the control group by Dunnett's tests and in the control group, significant ($P \leq 0.05$) linear dose trend effects based on contrast comparisons.

The necropsy body weights and the absolute and relative organ weights of rats are listed in Table F2. Significant dose-related decreases in body weights at necropsy and in absolute organ weights of brain, liver, heart, spleen, and thymus were observed for Aloe vera whole leaf extract-treated male and female rats and of the lung and kidney for female rats. Significant pairwise comparison tests results with controls were observed primarily in rats administered the 2.0 and 3.0% Aloe vera whole leaf extract. Significant dose-related increases were observed in the relative organ weights of brain and heart, suggesting that the decreased body weights of rats administered the Aloe vera whole leaf and episodic diarrhea may have contributed to dehydration in these animals, despite their increased water consumption.

Hematology and clinical chemistry was performed on blood samples collected from rats in the metabolism study. As observed in the 14-day studies, a greater than two-fold increase was observed in leukocytes counts in blood collected from male and female rats treated with the 2.0% Aloe whole leaf extract and compared to the controls. Leukocyte differential counts showed that the percentage of neutrophils accounted for a four-fold increase in males and a two-fold increase in females when compared to control levels, suggesting the presence of an inflammatory response. Erythrocyte counts were also elevated by the Aloe vera whole leaf extract. Clinical chemistry values for cholesterol and albumin were lower in 2% male and female rats, and creatinine levels were increased above control levels. In 2% female rats, blood urea nitrogen was elevated; however, values were within stated references ranges for laboratory rats (Table E2).

Table E4 lists the 24 h urine chemistry for days 30, 60, and 90 of the 13-week metabolism study in rats. Decreased urine production in the presence of increased water consumption was observed in male and female rats treated with the 2.0% Aloe vera whole leaf extract when compared to control levels on days 30, 60, and 90. Protein and glucose levels were increased in male and female rats at 30 days and at 60 day in male only. Urine 24 h creatinine levels were significantly decreased at 30, 60, and 90 day collections in male rats and at 30 and 60 days in female rats.

The transit times of carmine red dye in the gastrointestinal tract of rats in the 13-week metabolism study are shown in Table G2. Decreases in the gastrointestinal transit time of carmine red dye were observed at weeks 4, 8 and 12 in

male and female rats treated with the Aloe vera whole leaf extract in pairwise comparison tests with control rats. Transit times of carmine red at week 12 were 11.5 and 11.0 h in male and female control rats, respectively, and were 4.3 and 6.2 h in male and female rats, respectively, administered 2.0% Aloe vera whole leaf extract (Table G2).

The results of pathology examinations were based on 47 male and 47 female rats allocated to subchronic study and 12 male and 12 female rats allocated to the metabolism study. A dosing error resulted in the removal of one male rat from the 1.0% Aloe vera whole leaf dose group, and one female rat from the 3.0% dose group was found dead and advanced autolysis precluded its examination by histopathology.

There were no gross observations noted in any groups of rats that were treatment related, with the exception of an increased incidence of mesenteric lymph node enlargement in the 2.0 and 3.0% Aloe vera whole leaf treatment groups.

Histological evaluations found no incidence of neoplasms in any of the rats in this study. Nonneoplastic changes observed by histopathology related to the administration of the Aloe vera whole leaf extract were found primarily in large intestine, where goblet cell hyperplasia was detected. Lymphoid hyperplasia of the mesentery lymph nodes was prevalent in all Aloe vera whole leaf dose groups, but the severity was markedly increased in the 2.0 and 3.0% dose groups. The incidence and severity of goblet cell hyperplasia is tabulated in Table 4. The incidence rates of goblet cell hyperplasia of the cecum and colon was 100% in male and female rats administered the 2.0 and 3.0% doses of Aloe vera whole leaf extract. Figure 3 depicts the colon of a control animal (Panel A) and the changes that were observed in the rat colon following the daily administration of 1.0, 2.0, or 3.0% Aloe vera whole leaf extract in the drinking water for 13-weeks (Panels B, C, and D, respectively).

Exposure Concentration Selection Rationale: At concentrations of 2.0% or higher, the Aloe vera whole leaf induced significantly shorter gastrointestinal transit times and contributed to depressed body weights. The exposure concentrations of the Aloe vera whole leaf for the 2-year studies in rats were set just below the concentrations that

were known to induce significant body weight and gastrointestinal changes in the 13-week studies, 0.0%, 0.5%, 1.0%, and 1.5% (wt/wt).

TABLE 4
Incidence and Severity of Goblet Cell Hyperplasia of Rats in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	1%	2%	3%
Male				
Subchronic				
Cecum	0/12 (0.0%)[a]	6/11 (54.5%)	10/10 (100.0%)	7/7 (100.0%)
Severity[b]		2.2	2.2	3.0
Colon	0/12 (0.0%)	10/11 (90.9%)	10/10 (100.0%)	7/7(100.0%)
Severity		2.0	2.4	2.7
Rectum	0/12 (0.0%)		9/10 (90.0%)	4/7 (57.1%)
Severity			2.0	2.5
Metabolism				
Cecum	0/12 (0.0%)		12/12 (100.0%)	
Severity			1.7	
Ascending Colon	0/12 (0.0%)		12/12 (100%)	
Severity			1.8	
Transverse Colon	0/12 (0.0%)		12/12 (100%)	
Severity			2.5	
Descending Colon	0/12 (0.0%)		12/12 (100.0%)	
Severity			1.5	
Female				
Subchronic				
Cecum	1/12 (8.3%)	6/12 (50.0%)	8/8 (100.0%)	4/4 (100.0%)
Severity	1.0	1.5	2.3	2.3
Colon	0/12 (0.0%)	8/12 (66.7%)	8/8 (100.0%)	4/4 (100.0%)
Severity		1.5	2.5	3.0
Rectum	0/12 (0.0%)		7/8 (87.5%)	3/4 (75.0%)
Severity			1.6	1.7
Metabolism				
Cecum	0/12 (0.0%)		8/9 (88.9%)	
Severity			1.8	
Ascending Colon	0/12 (0.0%)		9/9 (100.0%)	
Severity			1.6	
Transverse Colon	0/12 (0.0%)		9/9 (100.0%)	
Severity			2.4	
Descending Colon	0/12 (0.0%)		6/9 (66.7%)	
Severity			1.5	

[a] Incidence reported as number of lesion bearing animals over total number of animals examined in the group
[b] Nonneoplastic lesions were graded for severity as 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked).

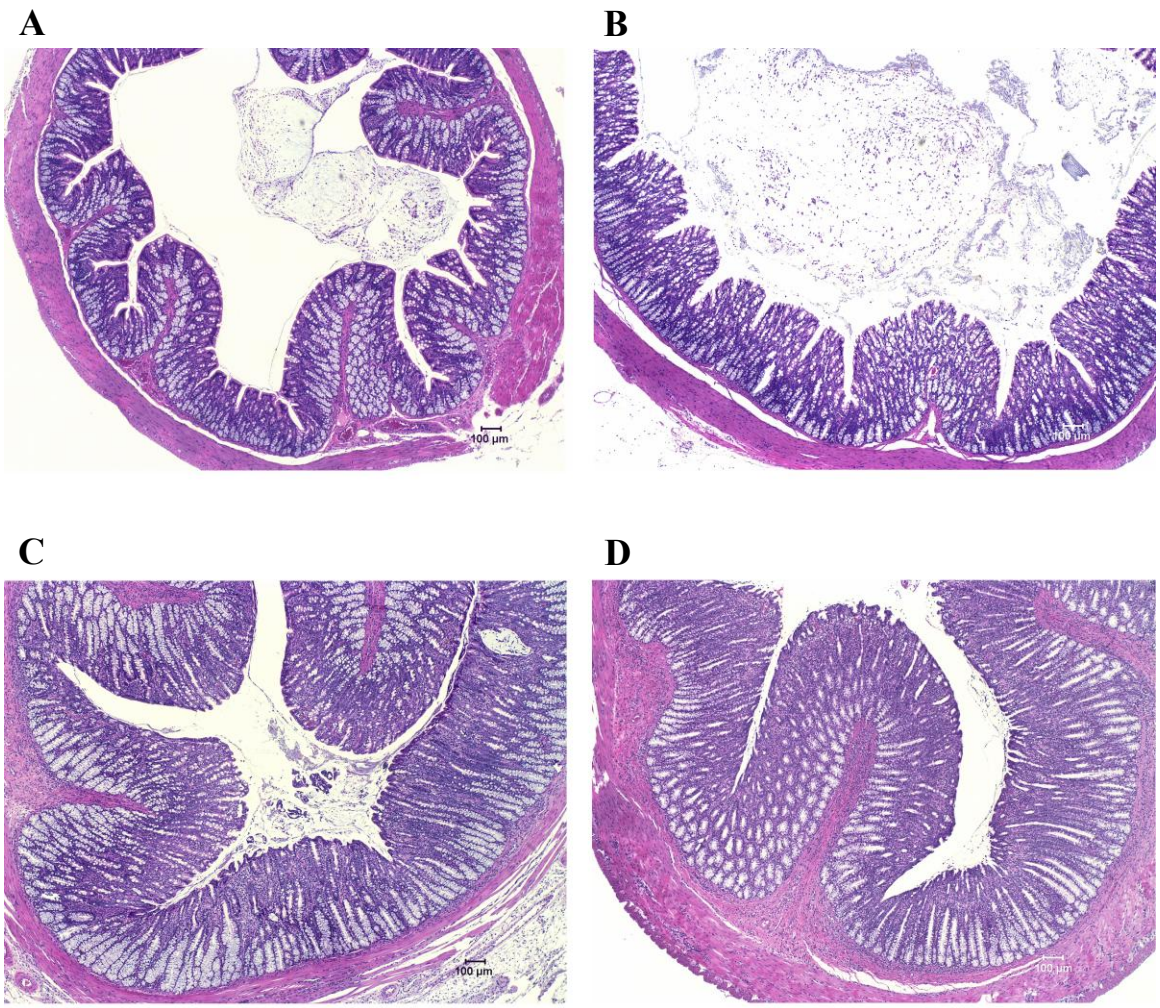


FIGURE 3
Goblet Cell Hyperplasia in the Colons of Rats in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract
Colon sections from rats treated with Aloe vera whole leaf extract at A) 0.0%, B) 1.0%, C) 2.0%, and D) 3.0%. Magnification is 4x.

2-YEAR STUDY

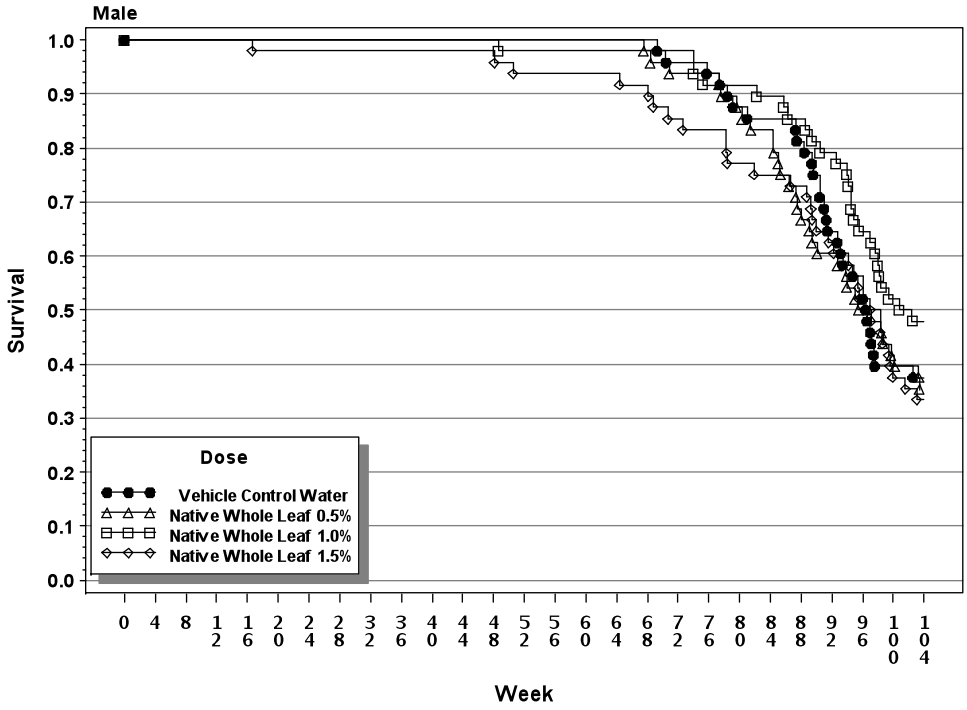
Survival and Cause of Death

The disposition, Kaplan-Meier estimates of mean survival times (weeks), and 2-year survival probabilities for male and female rats are shown in Table 5 and are graphically depicted in the Kaplan-Meier survival curves in Figure 4. Survival of all exposed groups of male rats was similar to that of the controls. Results of the Cox proportional hazard analysis showed a significant dose related decrease in the survival of female rats. When compared to controls, female rats that received the 1.5% Aloe vera whole leaf dosed water had a marginally lower rate of survival.

TABLE 5
Survival and Disposition of Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0.0%	0.5%	1.0%	1.5%
Male				
Animals initially in study	48	48	48	48
Moribund	31	26	27	29
Natural deaths	2	5	2	4
Animals surviving to study termination	15	17	19	15
Mean survival (weeks)[a]	94.4	93.0	96.3	90.0
Hazard ratio for survival[b]	1.00	0.97	0.76	1.06
Survival analysis[c]	0.925	0.906	0.291	0.823
Female				
Animals initially in study	48	48	48	48
Moribund	16	17	19	22
Natural deaths	2	0	5	6
Animals surviving to study termination	30	31	24	20
Mean survival (weeks)	95.7	97.6	94.6	91.9
Hazard ratio for survival	1.00	0.93	1.48	1.84
Survival analysis	0.017*	0.836	0.212	0.044*

[a]Kaplan-Meier estimates of mean survival time.
[b]Results of the Cox Proportional Hazards analysis.
[c]Results of the Cox Proportional Hazards trend tests are under the column for the control groups, and the results of pairwise comparison tests with the controls are under the columns for the exposed groups. Significant effects ($P < 0.05$) are signified by "*".



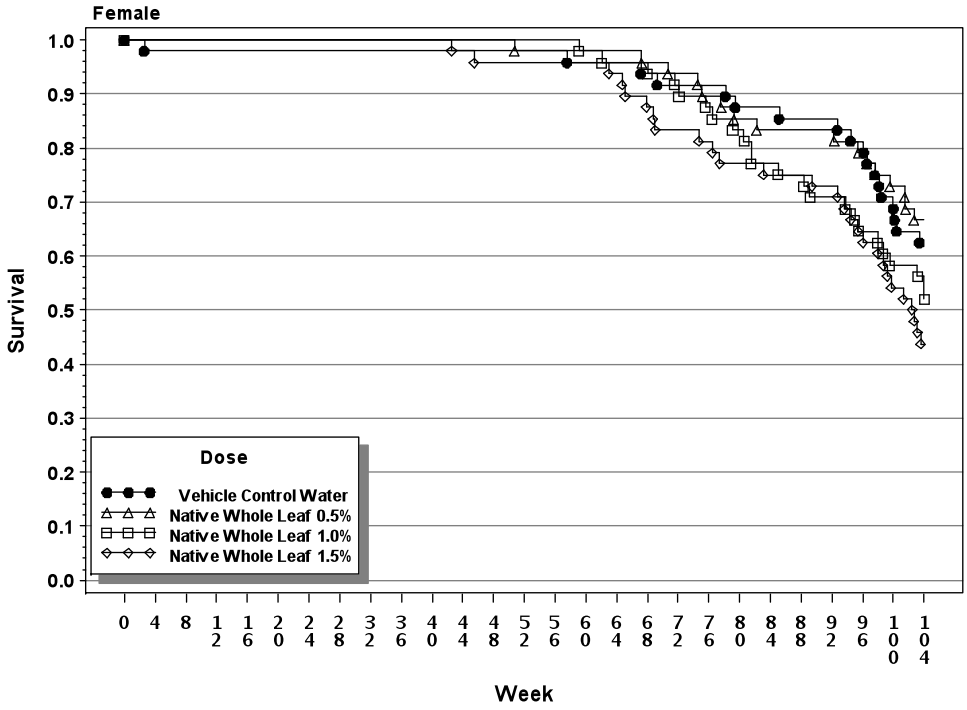


FIGURE 4
Kaplan-Meier Survival Curves for Rats in the
2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

The occurrences of intestinal lesions contributed to the cause of death in the two highest Aloe vera whole leaf dose groups of male and female rats. Dilatation of the cecum was the attributive cause of death for 8 and 12 female rats respectively, in the 1.0% and 1.5% Aloe vera whole leaf groups.

Body Weights and Feed and Water Consumption

The mean body weights of rats throughout the 2-year study are shown in four week intervals for males in Table 6 and females in Table 7 and are graphically represented in Figure 5. Mean body weights of male rats showed significant dose-related decreases. Depressed body weight gains were observed beginning at week 4 of the study, and this trend continued until study week 92, after which all male groups had similar body weights (Table 6). Mean body weights of the 1.5% Aloe vera whole leaf group of male rats ranged from 85.5 – 89.7% of control values between weeks 4 and 84 of the study. Significant dose-related decreases in mean body weights were observed at every four week interval throughout the study in female rats (Table 7 and Figure 5). Mean body weights of the 0.5%, 1.0%, and 1.5% Aloe vera whole leaf groups of female rats were 96.3%, 86.4%, and 79.5%, respectively, of the control group at the end of the study.

With few exceptions, feed consumption of the 0.5% and 1.0% groups of male and female rats were similar to controls. Significantly lower feed consumption values were observed for the 1.5% Aloe vera whole leaf treatment groups of male and female rats in pairwise comparison tests with controls. The overall mean feed consumptions relative to control values for the 1.5% Aloe vera whole leaf groups were 94.1% in male rats and 90.9% in female rats. The lower intakes of feed by the 1.5% Aloe vera whole leaf groups of male and female rats may explain, at least in part, the decrease in mean body weights observed in the same groups of rats.

Significantly notable increases in mean daily water consumptions were observed for male rats beginning at week 4 of the study, and dose-related increases in water consumptions continued until study week 92 (Table J1). With few exceptions, significant dose-related increases in water consumption were observed for each four week interval throughout the study in female rats (Table J2). It is noteworthy to mention that the decrease in body weights of rats followed a strikingly similar, albeit opposing, response pattern as that for water consumption of male and female

rats. Mean daily water consumptions of the 0.5%, 1.0% and 1.5% Aloe vera whole leaf dose groups of male rats for the 2 year study were 22.48, 26.98, and 31.0 g, respectively, and that of female rats from the same treatment groups were 18.16, 19.08, and 20.35 g, respectively. Mean overall water consumptions relative to control values were 103.0%, 123.9% and 142.4% for males and 101.3%, 106.5%, and 113.5% for females in the 0.5%, 1.0%, or 1.5% Aloe vera whole leaf treatment groups, respectively.

Drinking water concentrations of 0.5%, 1.0%, or 1.5% (wt/wt) of Aloe vera whole leaf resulted in average daily doses of approximately 0.2, 0.6, or 1.1 g Aloe vera whole leaf/kg of body weight for male rats and 0.3, 0.7, or 1.3 g Aloe vera whole leaf/kg body weight for female rats. The average aloin A and aloe-emodin content of the Aloe vera whole leaf test material was 6.40 and 0.071 mg/g, respectively. Based on average water consumption values, the daily doses of aloin A consumed by the 0.5%, 1.0%, and 1.5% Aloe vera whole leaf groups of rats were approximately 1.6, 4.0, and 7.2 mg/kg body weight for males and 2.1, 4.7, and 8.2 mg/kg body weight for female rats, respectively. The average daily doses of aloe-emodin consumed by the 0.5%, 1.0%, and 1.5% Aloe vera whole leaf groups of rats were approximately 17.6, 43.9, and 80.0 μg/kg body weight for males and 23.3, 52.1, and 90.9 μg/kg body weight for females, respectively.

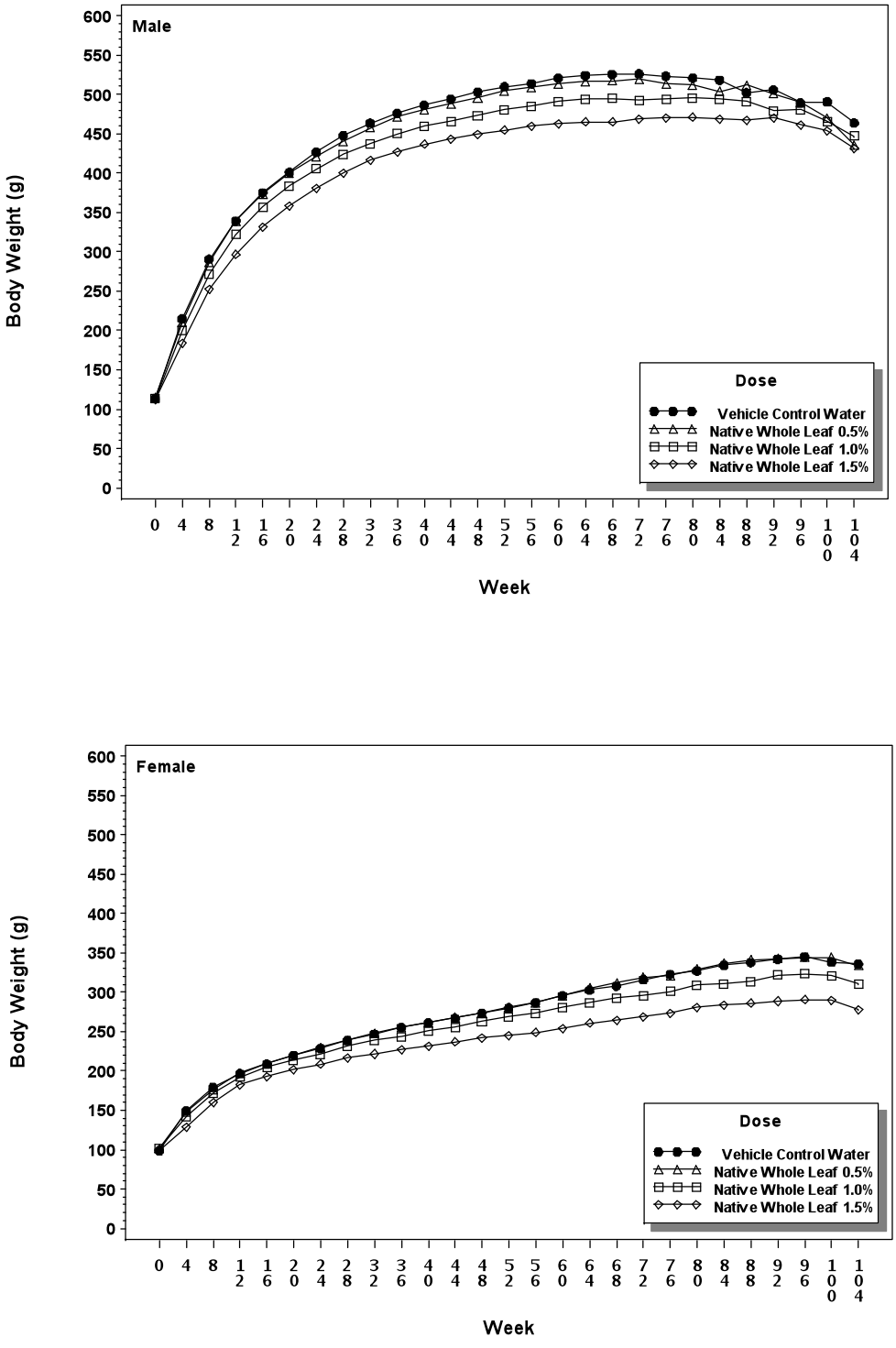


FIGURE 5
Growth Curves for Rats in the
2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

Weeks on Study	0.0 %		0.5%			1.0%			1.5%		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	214.9	48	211.3	98.3	48	200.0	93.1	48	183.8	85.5	48
8	290.7	48	286.9	98.7	48	272.5	93.8	48	252.3	86.8	48
12	339.5	48	338.7	99.8	48	321.8	94.8	48	296.5	87.3	48
16	375.3	48	373.9	99.6	48	356.8	95.1	48	331.1	88.2	48
20	401.4	48	399.8	99.6	48	383.5	95.5	48	357.1	89.0	47
24	426.7	48	421.5	98.8	48	405.6	95.0	48	379.5	88.9	47
28	447.7	48	440.8	98.5	48	424.2	94.8	48	398.9	89.1	47
32	463.6	48	457.6	98.7	48	438.0	94.5	48	415.2	89.6	47
36	476.0	48	471.4	99.1	48	450.1	94.6	48	426.4	89.6	47
40	486.3	48	480.8	98.9	48	459.3	94.4	48	436.0	89.7	47
44	494.2	48	487.9	98.7	48	465.7	94.2	48	442.2	89.5	47
48	503.0	48	495.9	98.6	48	473.2	94.1	48	448.6	89.2	46
52	509.7	48	504.6	99.0	48	479.9	94.1	47	453.8	89.0	45
56	514.0	48	508.7	99.0	48	484.3	94.2	47	458.8	89.3	45
60	520.7	48	513.5	98.6	48	490.1	94.1	47	462.4	88.8	45
64	524.2	48	516.9	98.6	48	493.6	94.2	47	464.5	88.6	45
68	525.5	48	516.8	98.3	47	494.1	94.0	47	464.6	88.4	43
72	525.1	46	516.9	98.4	45	492.2	93.7	47	466.1	88.8	41
76	523.0	45	512.0	97.9	45	491.0	93.9	44	464.5	88.8	40
80	520.2	42	509.1	97.9	42	492.5	94.7	44	463.0	89.0	37
84	515.4	41	494.8	96.0	40	489.1	94.9	43	460.6	89.4	36
88	497.1	39	488.5	98.3	32	482.5	97.1	41	458.0	92.1	35
92	486.7	31	477.6	98.1	29	473.2	97.2	38	454.7	93.4	29
96	461.5	25	469.8	101.8	24	467.2	101.2	31	448.1	97.1	25
100	443.8	19	450.9	101.6	19	448.4	101.1	25	434.9	98.0	18
104	409.9	18	410.3	100.1	17	423.8	103.4	21	408.7	99.7	16
Mean for weeks											
4-104	457.5		452.2	98.8		436.6	95.4		412.7	90.2	

TABLE 7
Mean Body Weights and Survival of Female Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

Weeks on Study	0.0 %		0.5%			1.0%			1.5%		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	149.7	48	147.9	98.8	48	142.6	95.3	48	128.9	86.1	48
8	179.3	47	176.3	98.3	48	171.8	95.8	48	160.7	89.6	48
12	196.8	47	197.1	100.2	48	192.2	97.7	48	182.2	92.6	48
16	209.1	47	209.7	100.3	48	204.6	97.8	48	193.0	92.3	48
20	220.3	47	219.3	99.6	48	213.9	97.1	48	201.6	91.5	48
24	228.9	47	230.0	100.5	48	221.5	96.8	48	208.8	91.2	48
28	238.9	47	238.8	100.0	48	231.2	96.8	48	216.4	90.6	48
32	247.3	47	248.1	100.3	48	238.9	96.6	48	221.9	89.7	48
36	255.7	47	255.7	100.0	48	244.0	95.5	48	227.2	88.9	48
40	261.0	47	261.3	100.1	48	250.6	96.0	48	231.5	88.7	48
44	267.0	47	268.1	100.4	48	255.9	95.8	48	237.1	88.8	47
48	273.5	47	273.6	100.0	48	263.0	96.1	48	241.7	88.4	46
52	280.1	47	280.8	100.3	47	268.9	96.0	48	244.7	87.3	46
56	287.0	47	286.8	99.9	47	273.7	95.4	48	248.6	86.6	46
60	295.5	46	296.1	100.2	47	280.5	94.9	47	254.0	86.0	46
64	302.6	46	305.6	101.0	47	286.0	94.5	46	259.4	85.7	45
68	308.6	45	312.3	101.2	46	291.9	94.6	45	262.3	85.0	42
72	315.4	44	319.9	101.4	45	294.6	93.4	43	264.8	84.0	40
76	322.3	44	322.6	100.1	43	298.9	92.7	42	268.6	83.3	39
80	327.0	42	327.7	100.2	41	306.3	93.7	40	274.4	83.9	37
84	334.1	42	334.1	100.0	40	307.9	92.2	37	279.5	83.7	36
88	336.9	41	338.6	100.5	40	308.5	91.6	36	281.3	83.5	36
92	341.4	41	340.5	99.7	40	312.7	91.6	34	283.2	83.0	35
96	343.5	38	339.6	98.9	38	310.0	90.3	31	282.6	82.3	30
100	339.8	32	334.4	98.4	35	304.0	89.4	28	280.7	82.6	26
104	333.6	30	321.3	96.3	32	288.3	86.4	25	265.2	79.5	20
Mean for weeks											
4-104	276.7		276.4	99.9		260.1	94.0		238.5	86.2	

Pathology and Histopathology

Clinical signs observed in groups of male and female rats related to Aloe vera whole leaf administration were hypoactivity, thinness, urine staining of the fur, hunched posture, abdominal masses, and diarrhea.

Complete necropsies were performed on rats that died naturally, that were removed from the study as moribund prior to terminal sacrifice, or that survived until scheduled terminal sacrifice. Gross lesions related to treatment with the Aloe vera whole leaf extract were primarily restricted to the large intestine. The results of pathology examinations were based on 192 male and 192 female rats allocated to the study. The results reported for the pathology and histopathology data in the following sections describe the statistically significant or biologically relevant changes in the incidences of neoplasms and nonneoplastic lesions in rats.

Neoplastic Findings

Aloe vera whole leaf extract treatment-related neoplasms occurred primarily in the large intestine of the rat. In the subchronic (13-week) testing on the Aloe vera whole leaf extract, the colon was identified as a potential target organ of toxicity. Therefore, additional sampling sites of the rat intestinal tract than those examined routinely in carcinogenicity studies were examined by histopathology for the 2-year study, including the ileo-cecal-colic junction (referred to in the tables as proximal colon), the cecum, and the ascending, transverse, and descending colon site sections (NTP, 2006).

Large Intestine: Histological identification of adenomas of the large intestine were either as pedunculated nodules, polyploid masses that protruded into the intestinal lumen, or sessile lesions that caused thickening of the intestinal wall (Figure 6 A). Epithelial cells within adenomas were well-differentiated and resembled cells in adjacent hyperplastic mucosal epithelium but formed distorted, glandular arrangements often with mild compression of adjacent mucosa. Diagnosis of carcinoma was based on invasion of the stroma of the stalk into the submucosa and/or muscularis of the intestinal wall and anaplastic changes in the neoplastic epithelial cells, including hyperchromatic staining and distortion of cellular size and shape (Figure 6B).

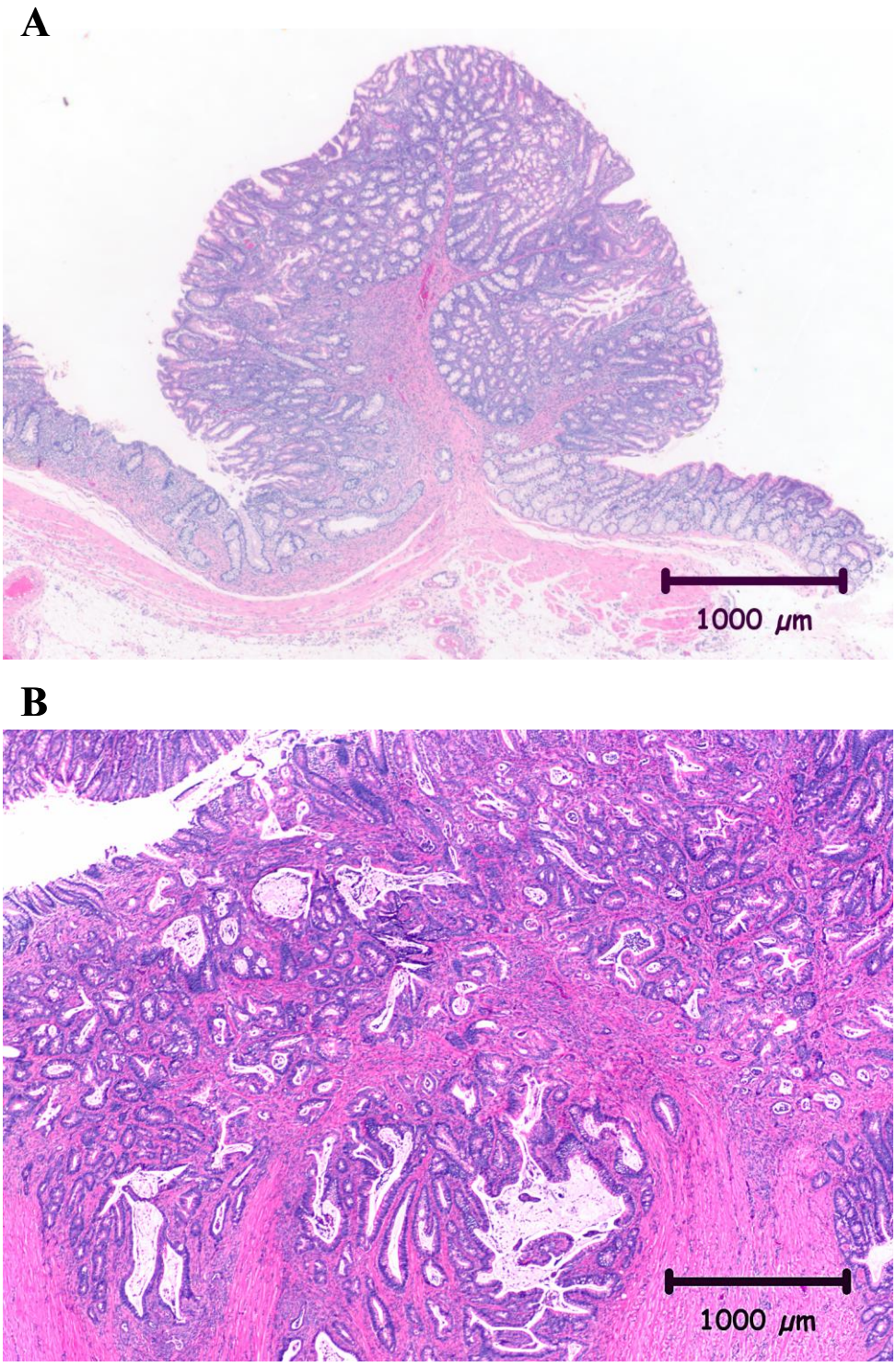


FIGURE 6
Adenoma and Carcinoma in the Large Intestines of Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract
Large intestine sections from rats treated with 1.5% Aloe vera whole leaf extract. A) Adenoma in a female rat. B) Carcinoma in a male rat.

Tables 8, 9, A2, and B2 summarize the observed and age-adjusted incidences of neoplasms and associated statistical results as related to the administration of the Aloe vera whole leaf extract. There were significant dose-trend increases in the incidences of adenomas and carcinomas of the proximal and ascending colon and increased incidences of adenomas of the cecum and transverse colon in male rats. When compared to controls, significantly higher incidences of adenomas of the proximal and ascending colon and cecum were observed for the 1.0% and 1.5% Aloe vera whole leaf extract male rats, and higher incidences of adenomas of the transverse colon were observed for the 1.0% group of males. Significantly higher incidences of carcinomas of the ascending colon were observed in male rats administered the 1.5% Aloe vera whole leaf in the drinking water for 2 years. The inclusion of all adenomas of the large intestine, all carcinomas of the large intestine, or the sum of adenomas and carcinomas of the large intestine resulted in significant dose-trend increases in the incidences for each of these categories of neoplasms, and significantly higher incidences were observed for the 1.0% and 1.5% Aloe vera whole leaf male rat groups when compared to the control groups (Table 8). The NCTR historical control incidence of cecum and colon adenomas or carcinomas in female and male F344/N male rats is 0/519 and 0/567, respectively.

Female rats had significant dose trend increases in the incidences of adenomas and carcinomas of the proximal colon, and increased incidences of adenomas of the ascending colon and cecum were observed (Table 9). The consumption of the 1.5% Aloe vera whole leaf in the drinking water for 2 years resulted in a significantly higher incidences of carcinomas of the proximal colon in female rats when compared to controls. In comparison tests with controls, significantly higher incidences of adenomas of the proximal colon were observed for the 1.0% and 1.5% female rats, and significantly higher incidences of adenomas of ascending colon and cecum were observed for females in the 1.5% Aloe vera whole leaf group. The inclusion of all adenomas of the large intestine, all carcinomas of the large intestine, or the sum of adenomas and carcinomas of the large intestine resulted in significant dose-trend increases in the incidences for each of these categories of neoplasms, and significantly increased incidences of adenomas and the sum of adenomas and carcinomas were found for the 1.0% and 1.5% Aloe vera whole leaf groups of female rats when compared to the control group. Compared to the control group, female rats administered the 1.5% Aloe vera whole leaf had significantly higher incidences of carcinomas of the large intestine (Table 9). The NCTR historical control incidence of cecum and colon adenomas or carcinomas in female and male F344/N rats is 0/527 and 0/623, respectively.

TABLE 8
Statistical Analysis of Neoplasms of the Large Intestine in Male Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	0.5%	1.0%	1.5%
Proximal Colon: Adenoma				
Overall rate[a]	0/44 (0%)***	0/44 (0%)	7/46 (15%)*	10/41 (24%)***
Proximal Colon: Carcinoma				
Overall rate	0/44 (0%)**	0/44 (0%)	4/46 (9%)	4/41 (10%)
Cecum: Adenoma				
Overall rate	0/46 (0%)***	0/45 (0%)	8/48 (17%)**	8/48 (17%)**
Cecum: Carcinoma				
Overall rate	0/46 (0%)	0/45 (0%)	1/48 (2%)	2/48 (4%)*
Ascending Colon: Adenoma				
Overall rate	0/47 (0%)***	0/47 (0%)	19/48 (40%)***	8/46 (17%)**
Ascending Colon: Carcinoma				
Overall rate	0/47 (0%)***	0/47 (0%)	4/48 (8%)	8/46 (17%)**
Transverse Colon: Adenoma				
Overall rate	0/47 (0%)*	0/47 (0%)	6/47 (13%)*	3/47 (6%)
Transverse Colon: Carcinoma				
Overall rate	0/47 (0%)	0/47 (0%)	1/47 (2%)	1/47 (2%)
All Adenomas				
Overall rate	0/47 (0%)	0/48 (0%)	26/48 (54%)	23/48 (48%)
Adjusted rate[b]	0%	0%	63.2%	59.8%
Terminal rate[c]	0/15 (0%)	0/17 (0%)	16/19 (84%)	10/15 (67%)
First incidence (days)	---[e]	---	597	488
Poly-3 test[d]	P<0.001	---	P=0.001	P<0.001
All Carcinomas				
Overall rate	0/47 (0%)	0/48 (0%)	10/48 (21%)	14/48 (29%)
Adjusted rate	0%	0%	24.9%	36.4%
Terminal rate	0/15 (0%)	0/17 (0%)	5/19 (26%)	4/15 (27%)
First incidence (days)	---	---	619	444
Poly-3 test	P<0.001	---	P=0.001	P<0.001
All Adenomas or Carcinomas[f]				
Overall rate	0/47 (0%)	0/48 (0%)	28/48 (58%)	31/48 (65%)
Adjusted rate	0%	0%	66.9%	74.2%
Terminal rate	0/15 (0%)	0/17 (0%)	16/19 (84%)	12/15 (80.0%)
First incidence (days)	---	---	597	444
Poly-3 test	P<0.001	---	P<0.001	P<0.001

[a] Number of neoplasm-bearing animals/number of animals examined.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at terminal kill.
[d] Beneath the control incidence the P value is associated with the trend test. Beneath the exposed group incidence the P values correspond to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. P-values that are significant are annotated with "*" to indicate $p \leq 0.05$, "**" to indicate $p \leq 0.01$, or "***" to indicate $p \leq 0.001$.
[e] Not applicable; no neoplasms in animal group.
[f] The historical incidence of large intestine adenoma or carcinoma in NCTR control male F344/N rats is 0/623.
Note: Proximal colon refers to the ileo-cecal-colic junction

TABLE 9
Statistical Analysis of Neoplasms of the Large Intestine in Female Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	0.5%	1.0%	1.5%
Proximal Colon: Adenoma				
Overall rate[a]	0/43 (0%)***	0/45 (0%)	4/42 (10%)*	5/39 (13%)*
Proximal Colon: Carcinoma				
Overall rate	0/43 (0%)**	0/45 (0%)	2/42 (5%)	4/39 (10%)*
Cecum: Adenoma				
Overall rate	0/47 (0%)***	0/48 (0%)	1/47 (2%)	6/48 (13%)*
Ascending Colon: Adenoma				
Overall rate	0/47 (0%)**	0/48 (0%)	1/46 (2%)	5/46 (11%)*
All Adenomas				
Overall rate	0/48 (0%)	0/48 (0%)	6/48 (13%)	13/48 (27%)
Adjusted rate[b]	0%	0%	15.7%	33.8%
Terminal rate[c]	0/30 (0%)	0/31 (0%)	5/24 (21%)	8/20 (40%)
First incidence (days)	---[e]	---	684	476
Poly-3 test[d]	P<0.001	---	P=0.011	P<0.001
All Carcinomas				
Overall rate	0/48 (0%)	0/48 (0%)	3/48 (6%)	4/48 (8%)
Adjusted rate	0%	0%	7.9%	10.9%
Terminal rate	0/30 (0%)	0/31 (0%)	3/24 (13%)	2/20 (10%)
First incidence (days)	---	---	729 (T)	679
Poly-3 test	P=0.005	---	P=0.105	P=0.047
All Adenomas or Carcinomas[f]				
Overall rate	0/48 (0%)	0/48 (0%)	8/48 (17%)	15/48 (31%)
Adjusted rate	0%	0%	20.9%	38.8%
Terminal rate	0/30 (0%)	0/31 (0%)	7/24 (29%)	9/20 (45)
First incidence (days)	---	---	684	476
Poly-3 test	P<0.001	---	P=0.002	P<0.001

(T) Terminal sacrifice.
[a] Number of neoplasm-bearing animals/number of animals examined.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at terminal kill.
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. P-values that are significant are annotated with "*" to indicate $p \leq 0.05$, "**" to indicate $p \leq 0.01$, or "***" to indicate $p \leq 0.001$.
[e] Not applicable; no neoplasms in animal group.
[f] The historical incidence of large intestine adenoma or carcinoma in NCTR control male F344/N rats is 0/527.
Note: Proximal colon refers to the ileo-cecal-colic junction

Incidence summaries of neoplasms in Tables A1 and B1 indicate that the carcinogenic response to the 2-year administration of Aloe vera whole leaf in the drinking water was considerably greater in 1.0% and 1.5% males than females. In the 1.0% Aloe vera whole leaf groups, the incidences of ascending colon adenomas, all adenomas of the large intestine, and the sum of adenomas and carcinomas of the large intestine were significantly higher in male rats compared to female rats ($p < 0.0001$, Fisher's exact two-sided test). The incidences of carcinomas of the large intestine and the sum of adenomas and carcinomas of the large intestine were significantly higher in the 1.5% Aloe vera whole leaf group of male rats compared to female rats from the same group ($p < 0.007$, Fisher's exact two-sided test). There were no neoplasms of the rectum in male or female rats, and metastasis of carcinomas from the large intestine was not observed in this study. There were no incidences of adenomas or carcinomas of the large intestine in control male and female rats.

Nonneoplastic Findings

Aloe vera whole leaf extract treatment-related nonneoplastic lesions occurred primarily in the large intestine and associated mesenteric lymph nodes. The observed and age-adjusted incidences and severity scores of nonneoplastic lesions are summarized for male and female rats in Tables 10 and 11, respectively.

Mucosal hyperplasia was a frequent finding in the large intestines of rats that consumed the Aloe vera whole leaf extract in the drinking water (Figure 7). The severities were greater and the incidences of mucosal hyperplasia were higher in the ascending and transverse colon compared to the descending colon sites of the large intestine of rats – the same sites that had increased incidences of neoplasms.

In male rats (Table 10), the administration of the Aloe vera whole leaf in the drinking water induced significant dose-related increasing trends in the incidences of mucosal hyperplasia of the proximal, ascending, transverse, and descending colon and cecum. In comparison to the control group, a significant treatment associated increase in the incidence of mucosal hyperplasia was observed for the proximal colon, the cecum, and the ascending, transverse, and descending colon and rectum of the large intestine at each dose level of the Aloe vera whole leaf. Degeneration and hyperplasia of mesenteric lymph nodes, and hyperplasia of the glandular stomach mucosa and the mucosa of the

small intestine were significantly increased in a dose-dependent manner. The administration of Aloe vera whole leaf in the drinking water of male rats induced significant treatment-related increases in the incidences of cecal dilatation. In comparison with the control group, significantly higher incidences of cecal dilatation were found for the Aloe vera whole leaf 1.0% and 1.5% dose groups of male rats.

The administration of Aloe vera whole leaf in the drinking water of female rats induced significant dose-related increasing trends in the incidences of mucosal hyperplasia of the colon (proximal, ascending, transverse, and descending and cecum) (Table 11). The incidences of mucosal hyperplasia in the colons of female rats were significantly higher than the control group at each dose level of the Aloe vera whole leaf and in the ceca significant pairwise comparison test results with controls were observed for the 1.0% and 1.5% Aloe vera whole leaf treatment groups. Significant dose related increases in the incidences of hyperplasia were also found in the mucosa of the glandular stomach, forestomach, small intestine, and rectum. Cecal dilatation was prevalent in the 1.0% and 1.5% Aloe vera whole leaf groups of female rats and significant dose related and pairwise comparison tests with control group animals were observed in the increased incidences of this lesion. Marked cecal dilatation was considered the cause of death or moribund sacrifice in a number of female rats in the two highest dose levels of Aloe vera whole leaf. Degeneration and atrophy of mesenteric lymph nodes and forestomach inflammation were also significantly increased in a dose-dependent manner in female rats.

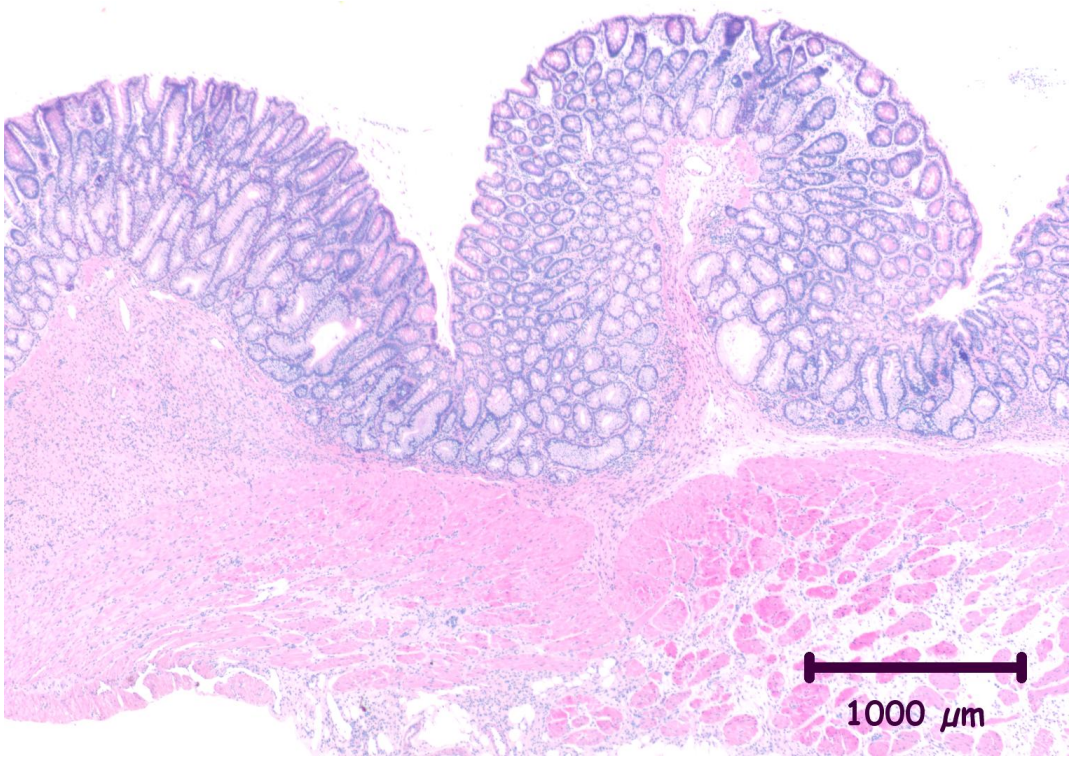


FIGURE 7
Mucosal Hyperplasia in the Large Intestine of a Male Rat in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract
Large intestine section from a male rat exposed to 1.5% Aloe vera whole leaf extract.

TABLE 10
Statistical Analysis of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	0.5%	1.0%	1.5%
Number Necropsied	48	48	48	48
Mesenteric Lymph Node				
Hyperplasia				
Overall rate[a]	0/47 (0.0%)	0/48 (0.0%)	1/48 (2.1%)	4/48 (8.3%)
Poly-3 test[b]	P=0.008 **	---	P=0.523	P=0.057
Average Severity	---	---	2	2.8
Cystic Degeneration				
Overall rate[a]	8/47 (17.0%)	11/48 (22.9%)	42/48 (87.5%)	41/48 (85.4%)
Poly-3 test[b]	P<0.001 ***	P=0.316	P<0.001 ***	P<0.001 ***
Average Severity	2.5	2.8	3.8	3.6
Glandular Stomach				
Mucosa Hyperplasia				
Overall rate	1/48 (2.1%)	12/47 (25.5%)	7/48 (14.6%)	11/48 (22.9%)
Poly-3 test	P=0.019 *	P<0.001 ***	P=0.040 *	P=0.002 **
Average Severity	2	2.3	2.4	2.4
Small Intestine				
Jejunum Mucosa Hyperplasia				
Overall rate	0/45 (0.0%)	1/44 (2.3%)	2/46 (4.3%)	3/46 (6.5%)
Poly-3 test	P=0.049 *	P=0.492	P=0.256	P=0.111
Average Severity	---	2	2	2
Large Intestine				
Proximal Colon Mucosa Hyperplasia				
Overall rate	0/44 (0.0%)	29/44 (65.9%)	36/46 (78.3%)	32/41 (78.0%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	---	2	2.5	3
Cecum Mucosa Hyperplasia				
Overall rate	0/46 (0.0%)	13/45 (28.9%)	24/48 (50.0%)	25/48 (52.1%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	---	1.5	1.9	2.4
Ascending Colon Mucosa Hyperplasia				
Overall rate	0/47 (0.0%)	30/47 (63.8%)	38/48 (79.2%)	32/46 (69.6%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	---	1.9	2.8	3.2
Transverse Colon Mucosa Hyperplasia				
Overall rate	0/47 (0.0%)	30/47 (63.8%)	42/47 (89.4%)	34/47 (72.3%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	---	1.8	2.2	2.4
Descending Colon Mucosa Hyperplasia				
Overall rate	0/47 (0.0%)	17/46 (37.0%)	31/46 (67.4%)	30/47 (63.8%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	---	1.5	1.6	1.7
Rectum Mucosa Hyperplasia				
Overall rate	0/47 (0.0%)	1/47 (2.1%)	1/48 (2.1%)	4/48 (8.3%)
Poly-3 test	P=0.022 *	P=0.495	P=0.515	P=0.055
Average Severity	---	1.0	2.0	2.0
Cecum Dilatation				
Overall rate	1/46 (2.2%)	0/45 (0.0%)	8/48 (16.7%)	17/48 (35.4%)
Poly-3 test	P<0.001 ***	P=0.512N	P=0.022 *	P<0.001 ***
Average Severity	5.0	---	5.0	5.0
Rectum Mucosa Hyperplasia				
Overall rate	0/47 (0.0%)	1/47 (2.1%)	1/48 (2.1%)	4/48 (8.3%)
Poly-3 test	P=0.022 *	P=0.495	P=0.515	P=0.055
Average Severity	---	1.0	2.0	2.0
All sites examined: Mucosa Hyperplasia				
Overall rate	0/47 (0.0%)	35/48 (72.9%)	48/48 (100%)	41/48 (85.4%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	---	2.2	3.1	3.4

[a] Number of lesion-bearing animals/number of animals examined.
[b] P-values under control group column represent results of linear trend tests with increasing dose levels of Aloe vera whole leaf. P-values under exposure group columns represent results of pairwise comparison tests with control groups. P-values that are significant are annotated with an "N" to indicate a negative statistic, "*" to indicate $p < 0.05$, "**" to indicate $p < 0.01$, or "***" to indicate $p<0.001$.

TABLE 11
Statistical Analysis of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	0.5%	1.0%	1.5%
Number Necropsied	48	48	48	48
Mesenteric Lymph Node				
Hyperplasia				
Overall rate[a]	0/46 (0.0%)	16/47 (34.0%)	40/48 (83.3%)	43/47 (91.5%)
Poly-3 test[b]	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	---	3.2	3.8	3.5
Cystic Degeneration				
Overall rate[a]	0/46 (0.0%)	16/47 (34.0%)	40/48 (83.3%)	43/47 (91.5%)
Poly-3 test[b]	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	---	3.2	3.8	3.5
Forestomach				
Stomach Inflammation				
Overall rate	0/48 (0.0%)	0/48 (0.0%)	4/48 (8.3%)	3/48 (6.3%)
Poly-3 test	P=0.013 *	---	P=0.056	P=0.1
Average Severity	---	---	3.3	3.3
Stomach Hyperplasia				
Overall rate	1/48 (2.1%)	7/48 (14.6%)	10/48 (20.8%)	9/48 (18.8%)
Poly-3 test	P=0.004 **	P=0.031 *	P=0.004 **	P=0.005 **
Average Severity	3.0	2.6	2.4	2.3
Glandular Stomach				
Mucosa Hyperplasia				
Overall rate	0/48 (0.0%)	1/48 (2.1%)	3/48 (6.3%)	3/48 (6.3%)
Poly-3 test	P=0.032 *	P=0.502	P=0.112	P=0.099
Average Severity	---	2.0	2.3	2.0
Small Intestine				
Ileum Mucosa Hyperplasia				
Overall rate	0/47 (0.0%)	2/48 (4.2%)	2/43 (4.7%)	6/44 (13.6%)
Poly-3 test	P=0.003 **	P=0.241	P=0.21	P=0.009 **
Average Severity	---	2.5	1.5	2.5
Large Intestine				
Proximal Colon				
Inflammation				
Overall rate	0/43 (0.0%)	2/45 (4.4%)	11/42 (26.2%)	8/39 (20.5%)
Poly-3 test	P<0.001 ***	P=0.243	P<0.001 ***	P=0.002 **
Average Severity	---	1.5	2.4	2.3
Mucosa Hyperplasia				
Overall rate	0/43 (0.0%)	30/45 (66.7%)	33/42 (78.6%)	32/39 (82.1%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	---	2.1	2.5	2.5
Cecum Mucosa Hyperplasia				
Overall rate	0/47 (0.0%)	4/48 (8.3%)	17/47 (36.2%)	27/48 (56.3%)
Poly-3 test	P<0.001 ***	P=0.064	P<0.001 ***	P<0.001 ***
Average Severity	---	1.8	2.2	2.2
Cecum Dilatation				
Overall rate	0/47 (0.0%)	0/48 (0.0%)	9/47 (19.1%)	25/48 (52.1%)
Poly-3 test	P<0.001 ***	---	P=0.002 **	P<0.001 ***
Average Severity	---	---	5.0	5.0
Ascending Colon Mucosa Hyperplasia				
Overall rate	0/47 (0.0%)	40/48 (83.3%)	35/46 (76.1%)	39/46 (84.8%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	---	2.0	2.3	2.6
Transverse Colon Mucosa Hyperplasia				
Overall rate	0/47 (0.0%)	40/48 (83.3%)	33/46 (71.7%)	42/46 (91.3%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P=<0.001 ***
Average Severity	---	1.7	1.8	2.1
Descending Colon Mucosa Hyperplasia				
Overall rate	0/47 (0.0%)	17/48 (35.4%)	18/46 (39.1%)	27/47 (57.4%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	---	1.4	1.4	1.5

TABLE 11
Statistical Analysis of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Large Intestine (continued)				
Rectum Mucosa Hyperplasia				
Overall rate	0/48 (0.0%)	0/48 (0.0%)	0/47 (0.0%)	5/47 (10.6%)
Poly-3 test	P=0.001 **	---	---	P=0.023 *
Average Severity	---	---	---	1.6
All sites examined: Mucosa Hyperplasia				
Overall rate	0/48 (0.0%	42/48 (87.5%)	42/48 (87.5%)	45/48 (93.8%)
Poly-3 test	P=0.001 **	P=0.001 **	P=0.001 **	P=0.001 **
Average Severity	---	2.3	2.8	3.0

[a] Number of lesion-bearing animals/number of animals examined.
[b] P-values under control group column represent results of linear trend tests with increasing dose levels of Aloe vera whole leaf. P-values under exposure group columns represent results of pairwise comparison tests with control groups. P-values that are significant are annotated with an "N" to indicate a negative statistic, "*" to indicate $p < 0.05$, "**" to indicate $p < 0.01$, or "***" to indicate $p<0.001$.

MICE

14-Day STUDY

The 14-day range-finding study in mice was conducted in an identical manner as the range-finding study in rats. There were no early deaths of mice (Table 12).

Aloe vera gel extract. Mean body weights and water consumption of mice are shown for male and female Aloe vera gel treated mice on Table 12. Mean body weights and body weight gains did not differ from controls. Dose-related increased water consumption was observed at week 2 in male and female mice; however, only 2% mice had significantly higher consumption levels than those of controls. Feed consumption was similar to controls (Table I1). Organ weights were similar to controls (Table F1). The results of clinical chemistry analyses showed that total protein and albumin demonstrated significant differences from control values; however, all values were within published reference ranges for laboratory mice (Table E5). Urine chemistry (Table E7), and gastrointestinal trasit time (Table G3) were similar to controls. The absolute and relative-to-necropsy-body-weight organ weights of mice at study termination are shown for Aloe vera gel treatment groups in Table F3 A dose-related decrease in kidney weights were found in male mice, and the kidney weight of 3% male mice was significantly lower than that of controls.

Aloe vera decolorized whole leaf extract. Mean body weights and water consumption of mice are shown for male and female Aloe vera decolorized whole leaf treated mice on Table 12. Body weights of male and female mice did not differ significantly from controls throughout the study, and body weights of all treatment groups were > 90.0% of control values at termination. Mean water and feed consumptions of male and female mice are shown in Table 12 and Table I3, respectively. Due to technician error, baseline feed consumption data was not collected on six cages of female mice administered the Aloe vera decolorized whole leaf extract; therefore, mean feed consumption values for week 0 represent the consumption of one cage of four mice for each dose level. Mice that received the Aloe vera decolorized whole leaf extract showed no treatment-related increases or decreases in water consumption. The absolute and relative-to-necropsy-body-weight organ weights of mice at study termination are shown for Aloe vera decolorized whole leaf treatment groups in Table F3. Absolute and relative organ and necropsy body weights were

similar to those of controls. Hematology values and clinical chemistry levels were within published reference ranges for laboratory mice. Urine chemistry values were similar to controls, with the exception of a decrease in protein in 3% female mice (Table E7). Male mice demonstrated a dose-related decrease in gastrointestinal transit time at week 2, and 3% male mice had significantly shorter trasit time when compared to that of the control (Table G3). Female mice demonstrated dose-related decreases in transit times at week 1, but no differences from controls were noted at week 2.

Aloe vera whole leaf extract. Body weights of male and female mice did not differ significantly from controls throughout the study, and body weights of all treatment groups were > 90.0% of control values at termination (Table 12). Significant dose-related increases in water consumption were observed for Aloe vera whole leaf extract in male mice at weeks 1 and 2 of dosing and in female mice at week 2 of dosing. Feed consumptions did not differ from controls in any treatment groups during the study. The absolute and relative-to-necropsy-body-weight organ weights of mice at study termination are shown for Aloe vera whole leaf treatment groups in Table F3. There were no biologically significant organ weight changes observed in mice. Male mice in the 3% Aloe vera whole leaf group demonstrated a significant increase in the number of leukocytes and in the levels of creatinine when compared to those of controls, and 2% and 3% female mice demonstrated elevated glucose levels compared to that of controls (Table E5).

TABLE 12
Survival, Body Weights, and Water Consumption of Mice in the 14-Day Drinking Water Study of Aloe vera Extracts

Aloe vera Extract and Concentration (%)	Survival[a]	Mean Body Weight[b] (g)				Final Weight Relative to Controls (%)	Mean Water Consumption[c]		
		Day 0	Day 7	Day 14	Change		Week 0	Week 1	Week 2
Male									
Gel									
0	8/8	21.88 ± 0.57	23.81 ± 0.58	24.18 ± 0.87	2.30 ± 0.70		5.90 ± 0.35	5.55 ± 0.38*	5.98 ± 0.71*
0.5	8/8	22.83 ± 0.57	24.41 ± 0.58	24.43 ± 0.87	1.60 ± 0.70	101.03	5.55 ± 0.34	5.55 ± 0.38	5.29 ± 0.71
1	8/8	22.33 ± 0.57	24.24 ± 0.58	25.18 ± 0.87	2.85 ± 0.70	104.14	6.27 ± 0.34	6.10 ± 0.38	6.71 ± 0.71
1.5	8/8	21.88 ± 0.57	23.91 ± 0.58	24.71 ± 0.87	2.84 ± 0.70	102.22	6.58 ± 0.35	5.87 ± 0.38	6.28 ± 0.71
2	8/8	21.35 ± 0.57	23.70 ± 0.58	24.38 ± 0.87	3.03 ± 0.70	100.83	6.01 ± 0.34	6.69 ± 0.38	9.77 ± 0.74*
3	8/8	21.40 ± 0.57	22.58 ± 0.58	23.69 ± 0.87	2.29 ± 0.70	97.98	5.80 ± 0.34	6.75 ± 0.38	6.33 ± 0.71
Decolorized Whole Leaf									
0	8/8	23.11 ± 0.47	24.19 ± 0.58	24.35 ± 0.79	1.24 ± 0.57		6.18 ± 0.32	8.11 ± 1.02	5.74 ± 0.43
0.5	8/8	23.05 ± 0.47	24.66 ± 0.58	25.23 ± 0.79	2.18 ± 0.57	103.59	5.99 ± 0.32	6.19 ± 1.02	5.97± 0.43
1	8/8	22.18 ± 0.47	23.93 ± 0.58	23.24 ± 0.79	1.06 ± 0.57	95.43	5.25 ± 0.32	7.14 ± 1.02	6.22 ± 0.43
1.5	8/8	22.80 ± 0.47	23.73 ± 0.58	25.13 ± 0.79	2.33 ± 0.57	103.18	5.56 ± 0.32	6.54 ± 1.02	5.77 ± 0.43
2	8/8	22.15 ± 0.47	24.19 ± 0.58	24.36 ± 0.79	2.21 ± 0.57	100.05	5.30 ± 0.32	5.70 ± 1.02	6.27 ± 0.43
3	8/8	23.10 ± 0.47	25.53 ± 0.58	26.30 ± 0.79	3.20 ± 0.57	108.01	5.54 ± 0.32	5.85 ± 1.02	5.44 ± 0.44
Whole Leaf									
0	8/8	21.64 ± 0.33	23.94 ± 0.41	23.31 ± 0.73	1.68 ± 0.63		6.34 ± 0.43	6.15 ± 0.50*	6.24 ± 0.43*
0.5	8/8	22.54 ± 0.33	24.58 ± 0.41	24.50 ± 0.73	1.96 ± 0.63	105.09	6.32 ± 0.43	6.88 ± 0.50	7.36 ± 0.43
1	8/8	23.00 ± 0.33*	25.10 ± 0.41	24.75 ± 0.73	1.75 ± 0.63	106.17	6.15 ± 0.43	6.02 ± 0.50	6.88 ± 0.43
1.5	8/8	22.03 ± 0.33	24.50 ± 0.41	24.29 ± 0.73	2.26 ± 0.63	104.18	6.61 ± 0.45	7.62 ± 0.52	7.24 ± 0.46
2	8/8	22.29 ± 0.33	24.90 ± 0.41	25.15 ± 0.73	2.86 ± 0.63	107.88	6.14 ± 0.43	7.66 ± 0.50	7.49 ± 0.43
3	8/8	22.25 ± 0.33	24.36 ± 0.41	24.83 ± 0.73	2.58 ± 0.63	106.49	6.21 ± 0.43	7.52 ± 0.50	7.73 ± 0.43

TABLE 12
Survival, Body Weights, and Water Consumption of Mice in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

Aloe vera Extract and Concentration (%)	Survival[a]	Mean Body Weight[b] (g)				Final Weight Relative to Controls (%)	Mean Water Consumption[c]		
		Day 0	Day 7	Day 14	Change		Week 0	Week 1	Week 2
Female									
Gel									
0	8/8	17.79 ± 0.59*	19.96 ± 0.49	19.66 ± 0.62	1.88 ± 0.28		5.73 ± 0.36	4.94 ± 0.32	5.01 ± 0.85*
0.5	8/8	18.16 ± 0.59	19.71 ± 0.49	19.93 ± 0.62	1.76 ± 0.28	101.34	5.11 ± 0.36	4.84 ± 0.31	5.48 ± 0.85
1	8/8	17.69 ± 0.59	18.83 ± 0.49	19.70 ± 0.62	2.01 ± 0.28	100.19	5.28 ± 0.36	4.85 ± 0.31	5.63 ± 0.85
1.5	8/8	17.46 ± 0.59	19.21 ± 0.49	18.94 ± 0.62	1.48 ± 0.28	96.31	5.76 ± 0.37	5.70 ± 0.31	5.94 ± 0.85
2	8/8	17.90 ± 0.59	19.06 ± 0.49	19.93 ± 0.62	2.03 ± 0.28	101.34	5.24 ± 0.36	4.93 ± 0.31	8.83 ± 0.85*
3	8/8	17.79 ± 0.59	18.99 ± 0.49	19.36 ± 0.62	1.58 ± 0.28	98.47	5.10 ± 0.36	5.53 ± 0.31	7.03 ± 0.85
Decolorized Whole Leaf									
0	8/8	17.63 ± 0.43	18.91 ± 0.42	19.60 ± 0.58*	1.98 ± 0.35		5.04 ± 0.28	6.17 ± 0.91	4.99 ± 0.46
0.5	8/8	17.40 ± 0.43	18.03 ± 0.42	18.66 ± 0.58	1.26 ± 0.35	95.22	4.72 ± 0.28	5.04 ± 0.91	5.19 ± 0.46
1	8/8	17.79 ± 0.43	18.58 ± 0.42	19.45 ± 0.58	1.66 ± 0.35	99.23	4.76 ± 0.28	5.07 ± 0.91	4.99 ± 0.46
1.5	8/8	17.55 ± 0.43	18.31 ± 0.42	18.86 ± 0.58	1.31 ± 0.35	96.24	4.75 ± 0.28	7.22 ± 0.91	6.70 ± 0.46*
2	8/8	17.98± 0.43	18.88 ± 0.42	19.46 ± 0.58	1.49 ± 0.35	99.30	4.59 ± 0.28	5.50 ± 0.91	5.67 ± 0.46
3	8/8	17.81± 0.43	19.25 ± 0.42	19.21 ± 0.58	1.40 ± 0.35	98.02	4.82 ± 0.28	5.22 ± 0.91	5.04 ± 0.46
Whole Leaf									
0	8/8	18.65 ± 0.47	19.61 ± 0.35*	19.61 ± 0.69	0.96 ± 0.39		5.43 ± 0.50	4.87 ± 0.93	5.10 ± 1.08*
0.5	8/8	18.30 ± 0.47	18.94 ± 0.35	19.31 ± 0.69	1.01 ± 0.39	98.47	4.57 ± 0.48	5.07 ± 0.93	5.63 ± 1.08
1	8/8	18.25 ± 0.47	19.48 ± 0.35	19.39 ± 0.69	1.14 ± 0.39	98.85	4.79 ± 0.48	5.00 ± 0.93	6.14 ± 1.08
1.5	8/8	18.49 ± 0.47	19.70 ± 0.35	19.58 ± 0.69	1.09 ± 0.39	99.81	5.03 ± 0.48	8.04 ± 0.93	7.28 ± 1.08
2	8/8	18.63 ± 0.47	19.60 ± 0.35	19.69 ± 0.69	1.06 ± 0.39	100.38	4.65 ± 0.48	5.27 ± 0.93	9.67 ± 1.08*
3	8/8	18.25 ± 0.47	19.24 ± 0.35	19.00 ± 0.69	0.75 ± 0.39	96.88	4.74 ± 0.48	5.89 ± 0.93	7.50 ± 1.08

[a] Number of animals surviving at 14 days/number initially in group.
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.
* Signifies values that are significantly different ($P \leq 0.05$) from control group by Dunnett's test and in the control group, significant linear dose trend ($P \leq 0.05$) effects based on contrast comparisons.

The transit times of carmine red dye in the gastrointestinal tract of mice in the 14-day metabolism study are shown in Table G3. Dose-related decreases in the transit time of carmine red dye were observed at week 1 in male mice treated with Aloe vera whole leaf extract (Table G3). The urine chemistry for 24 h collections on days 5 and 12 of the 14-day metabolism studies in mice are listed in Table E7. No biologically significant changes were observed in the measured parameters.

The results of pathology examinations were based on the 72 male and 72 female mice allocated to the study. No neoplastic lesions were observed in any of the mice in this study. Several nonneoplastic lesions were observed in a few mice on this study; however, these were considered incidental in nature and demonstrated no treatment-related effects.

Exposure Concentration Selection Rationale: All mice survived the 14-day study with no treatment-related gross or microscopic lesions. Aloe vera whole leaf extract was selected as the test article for further study, since it contains all of the Aloe vera constituents. Based upon the activity of Aloe vera extracts in the 14-day study, doses selected for the subsequent 13-week study were 0, 1, 2, and 3% (wt/wt) of the Aloe vera whole leaf extract.

13-WEEK STUDY

All mice survived until the end of the study (Table 13). There were no significant differences between mean body weights of male and female mice that received the Aloe vera whole leaf extract and their control counterparts. Final body weights of male and female mice administered the Aloe vera whole leaf extract ranged from 96.19 – 101.27% of control values (Table 13).

Mean water and feed consumptions of male and female mice on the subchronic and metabolism studies are shown in Table 13 and Table I4, respectively. Water consumption patterns among male and female mice on the subchronic and metabolism studies are shown on Table 13. Compared to control animals, significantly higher water consumptions were observed for both male and female mice that received 2.0% Aloe vera whole leaf extract at days 30 and 60, and significant dose trend increases in water consumption were observed in female mice at day 90. Feed consumption among male and female mice administered the Aloe vera whole leaf extract did not differ from that of controls, although feed consumptions of female rats on the subchronic study showed a significant dose-related increase at week 4. There were no differences observed in the feed or water consumption patterns of male and female mice on the metabolism study.

The results of hematology and clinical chemistry analyses for mice in the 13-week drinking water study are shown on Table E6. Reported values were within published reference values for the laboratory mice and did not indicate any significant treatment-related differences.

The necropsy body weights and the absolute and relative organ weights are shown for male and female mice on the subchronic and metabolism studies on Table F4. There were no biologically significant differences from controls in body weights at necropsy or in the absolute and relative organ weights of male or female mice that received the Aloe vera whole leaf extract.

TABLE 13
Survival, Body Weights, and Water Consumption of Mice in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract

Concentration (%)	Survival[a]	Mean Body Weight[b] (g)					Final weight Relative to Controls (%)	Mean Water Consumption[c]			
		Day 0	Day 30	Day 60	Day 92	Change		Day 0	Day 30	Day 60	Day 90
Subchronic Study											
Male											
0	12/12	20.96 ±0.34	27.76 ± 0.36	31.52 ± 0.48	32.84 ±0.52	11.88 ±0.51		6.41 ±2.73	6.95 ± 0.53	6.69 ± 1.61*	7.72 ±1.7
1	12/12	20.58 ±0.34	27.83 ± 0.36	31.22 ± 0.48	31.77 ±0.52	11.18 ±0.51	96.73	11.92 ±2.73	11.24 ± 0.53*	12.65 ± 1.61	11.1 ±1.7
2	12/12	20.83 ±0.34	27.83 ± 0.36	31.19 ± 0.48	31.74 ±0.52	10.91 ±0.51	96.65	6.27 ±2.73	10.17 ± 0.53*	20.91 ± 1.61*	13.6 ±1.7
3	12/12	20.95 ±0.34	27.15 ± 0.36	30.33 ± 0.48	31.59 ±0.52	10.64 ±0.51	96.19	5.92 ±2.73	7.77 ± 0.53	9.86 ± 1.61	10.9 ±1.7
Female											
0	12/12	17.46 ±0.29	21.80 ± 0.30	23.73 ± 0.34	25.79 ±0.57	8.33 ±0.49		5.55 ±2.63	5.12 ± 0.59	6.33 ± 1.21	5.64 ±0.8*
1	12/12	17.44 ±0.29	21.55 ± 0.30	23.68 ± 0.34	25.76 ±0.57	8.32 ±0.49	99.87	6.08 ±2.63	9.10 ± 0.59*	9.96 ± 1.21	9.32 ±0.8*
2	12/12	17.38 ±0.29	21.29 ± 0.30	24.09 ± 0.34	26.03 ±0.57	8.64 ±0.49	100.90	10.49 ±2.63	9.01 ± 0.59*	13.57 ± 1.21*	9.04 ±0.8*
3	12/12	17.63 ±0.29	21.53 ± 0.30	24.14 ± 0.34	25.48 ±0.57	7.85 ±0.49	98.80	5.08 ±2.63	7.01 ± 0.59	6.83 ± 1.21	9.17 ±0.8*
Metabolism Study											
Male											
0	12/12	19.48 ±0.37	25.52 ± 0.48	30.43 ± 0.49	31.43 ±0.48	11.96 ±0.49		5.97 ±0.67	6.42 ± 0.48	7.81 ± 0.45	5.44 ±2.9
3	12/12	19.48 ±0.37	24.41 ± 0.48	29.18 ± 0.49	29.96 ±0.48	10.48 ±0.49	95.30	4.66 ±0.67	7.63 ± 0.48	9.31 ± 0.45	14.2 ±2.9
Female											
0	12/12	16.27 ±0.19	18.53 ± 0.24	22.28 ± 0.20	22.77 ±0.23	6.50 ±0.32		4.51 ±3.67	7.31 ± 1.29	6.13 ± 2.43	5.34 ±2.7
3	12/12	16.27 ±0.19	18.84 ± 0.24	22.03 ± 0.20	23.06 ±0.23	6.79 ±0.32	101.27	14.65 ±3.67	7.46 ± 1.29	17.76 ± 2.43	14.7 ±2.7

[a] Number of animal surviving until study termination/number of animals initially in group.
[b] Weights and weight changes are given as mean ± standard error of the mean.
[c] Water consumption is given as mean ± standard error of the mean and are expressed as grams per animal per day.
* Signifies values that are significantly different ($P \leq 0.05$) from the control group by Dunnett's tests and in the control group, significant ($P \leq 0.05$) linear dose trend effects based on contrast comparisons.

The transit times of carmine red dye in the gastrointestinal tract of male and female mice on the metabolism study are listed in Table G4. A transient increase in transit time relative to controls was observed at week-8 of the study in male mice administered the 3.0% Aloe vera whole leaf. No differences in the transit times of carmine red were observed at weeks 4 or 12 in male mice or at any time point in female mice.

Significant increases in 24 h urinary levels of creatinine and micro protein were observed when compared with control levels in male and female mice at weeks 8 and 12; however, values were within normal physiological ranges for laboratory rodents (Table E8).

A complete necropsy was performed on all mice on the subchronic and metabolism study that were alive at the end of the studies, as well as those that were removed from the studies due morbidity or early death, with the exception of one female mouse on the subchronic study that escaped from its cage just prior to euthanasia. The results of pathology examinations were based on 48 male and 47 female mice allocated to the subchronic study and 24 male and 24 female mice allocated to the metabolism study. There were no meaningful gross observations noted in any groups of mice that were treatment related.

Nonneoplastic changes related to the Aloe vera whole leaf extract administration primarily occurred in the large intestine and resulted in increased incidences and severities of goblet cell hyperplasia. The incidence and severity of goblet cell hyperplasia is tabulated in Table 14 for mice in both the subchronic and metabolism studies and shows that goblet cell hyperplasia of the colon was observed in greater than 90% of male and 58% of female mice administered the 3.0% Aloe vera whole leaf extract. Figure 8 depicts the colon of a control animal (Panel A) and the changes that were observed in the mouse colon following the daily administration of 1.0, 2.0, or 3.0% Aloe vera whole leaf extract in the drinking water for 13-weeks (Panels B, C, and D), respectively.

Exposure Concentration Selection Rationale: The results of 13-week studies suggested that the Aloe vera whole leaf extract at concentrations up to 3.0% (wt/wt) was well tolerated by mice. Goblet cell hyperplasia, with mucin

present in the lumen, and mesenteric lymph node hyperplasia were observed in the large intestines of mice administered the Aloe vera whole leaf. For the 2-year drinking water studies of Aloe vera whole leaf, mice received doses of 0.0%, 1.0%, 2.0%, or 3.0% (wt/wt).

TABLE 14
Incidence and Severity of Goblet Cell Hyperplasia of Mice in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	1%	2%	3%
Male				
Subchronic				
Cecum	0/12 (0.0%)[a]	1/12 (8.3%)	5/12 (41.6%)	9/12 (75.0%)
Severity[b]		1.0	1.2	1.0
Colon	0/12 (0.0%)	3/12 (25.0%)	9/12 (75.0%)	11/12 (91.7%)
Severity		1.0	1.4	1.1
Rectum	0/12 (0.0%)			4/11 (36.4%)
Severity				1.0
Metabolism				
Cecum	0/12 (0.0%)			2/12 (16.7%)
Severity				1.0
Ascending Colon	0/12 (0.0%)			11/12 (91.7%)
Severity				1.0
Transverse Colon	0/12 (0.0%)			11/12 (91.7%)
Severity				1.3
Descending Colon	0/12 (0.0%)			9/12 (75.0%)
Severity				1.0
Female				
Subchronic				
Cecum	0/12 (0.0%)	0/12 (0.0%)	0/11 (0.0%)	1/12 (8.3%)
Severity				1.0
Colon	0/12 (0.0%)	0/12 (0.0%)	4/11 (36.4%)	7/12 (58.3%)
Severity			1.0	1.1
Rectum	0/12 (0.0%)			5/11 (45.5%)
Severity				1.0
Metabolism				
Cecum	0/12 (0.0%)			0/12 (0.0%)
Severity				
Ascending Colon	0/12 (0.0%)			7/12 (58.3%)
Severity				1.1
Transverse Colon	0/12 (0.0%)			7/12 (58.3%)
Severity				1.1
Descending Colon	0/12 (0.0%)			4/12 (33.3%)
Severity				1.2

[a] Incidence reported as number of lesion bearing animals over total number of animals examined in the group
[b] Nonneoplastic lesions were graded for severity as 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked).

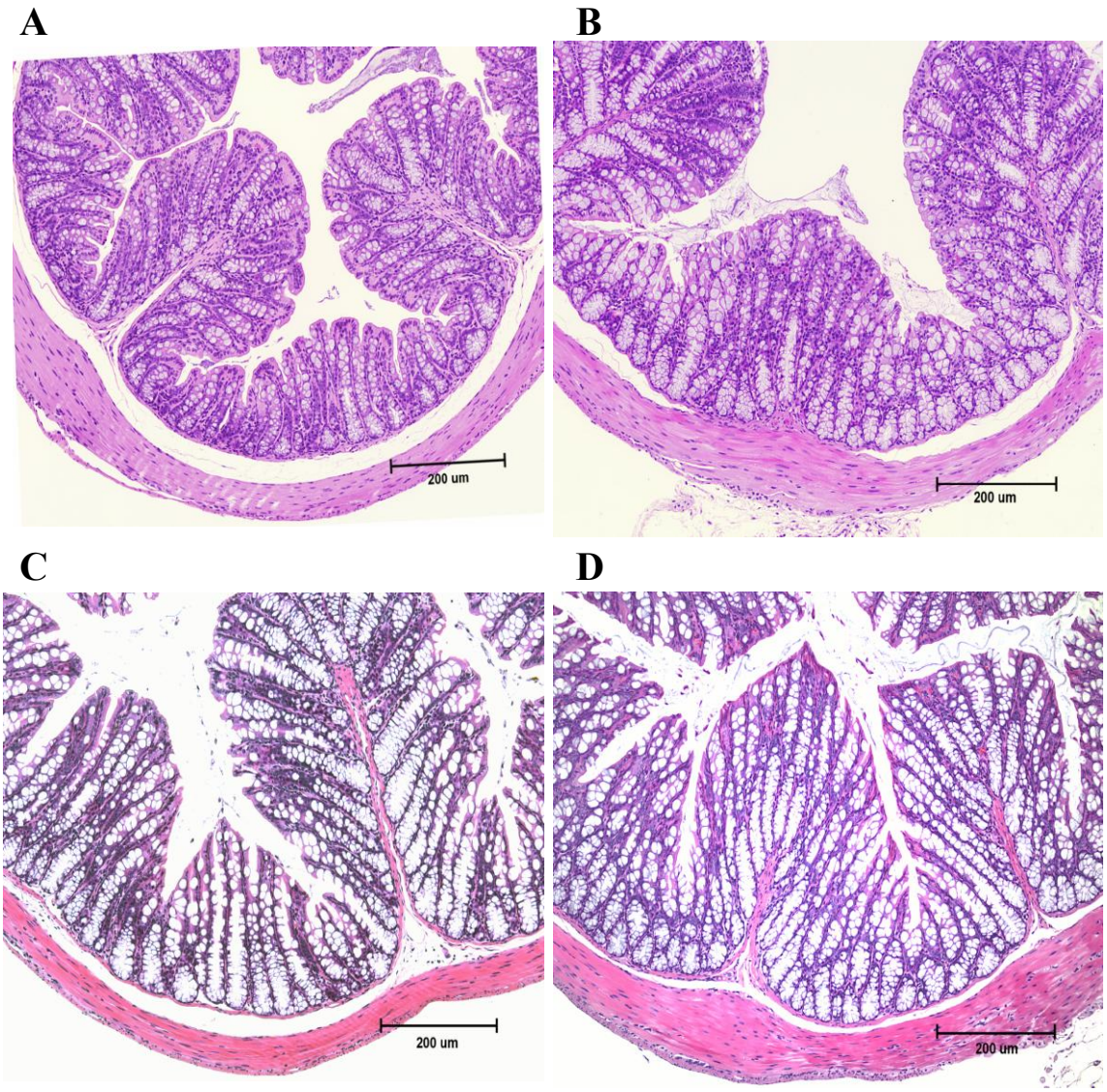


FIGURE 8
Goblet Cell Hyperplasia in the Colons of Mice in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract
Colon sections from mice treated with Aloe vera whole leaf extract at A) 0.0%, B) 1.0%, C) 2.0%, and D) 3.0%. Magnification is 10x.

2-YEAR STUDY

Survival and Cause of Death

The disposition, Kaplan-Meier estimates of mean survival times (weeks), and 2-year survival probabilities for male and female mice are shown in Table 15 and in the Kaplan-Meier survival curves (Figure 9). Survival of all exposed groups was similar to that of the controls.

TABLE 15
Survival and Disposition of Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0.0%	1.0%	2.0%	3.0%
Male				
Animals initially in study	48	48	48	48
Discard[a]		1		
Moribund	16	16	23	15
Natural deaths	1	3	4	5
Animals surviving to study termination	31	28	21	28
Mean survival (weeks)[b]	94.2	97.2	93.6	93.1
Hazard ratio for survival[c]	1.00	1.05	1.71	1.22
Survival analysis[d]	0.298	0.876	0.082	0.550
Female				
Animals initially in study	48	48	48	48
Discard	1			
Moribund	7	13	8	7
Natural deaths	5	5	4	7
Animals surviving to study termination	35	30	36	34
Mean survival (weeks)	101.2	98.3	101.0	97.1
Hazard ratio for survival	1.00	1.60	0.99	1.23
Survival analysis	0.915	0.206	0.987	0.606

[a]One animal erroneously removed as discard.
[b]Kaplan-Meier estimates of mean survival time.
[c]Results of the Cox Proportional Hazards analysis.
[d]The results of the Cox Proportional Hazards trend tests are under the control group column, and the results of pairwise comparison tests with the controls are under the columns for the exposed groups.

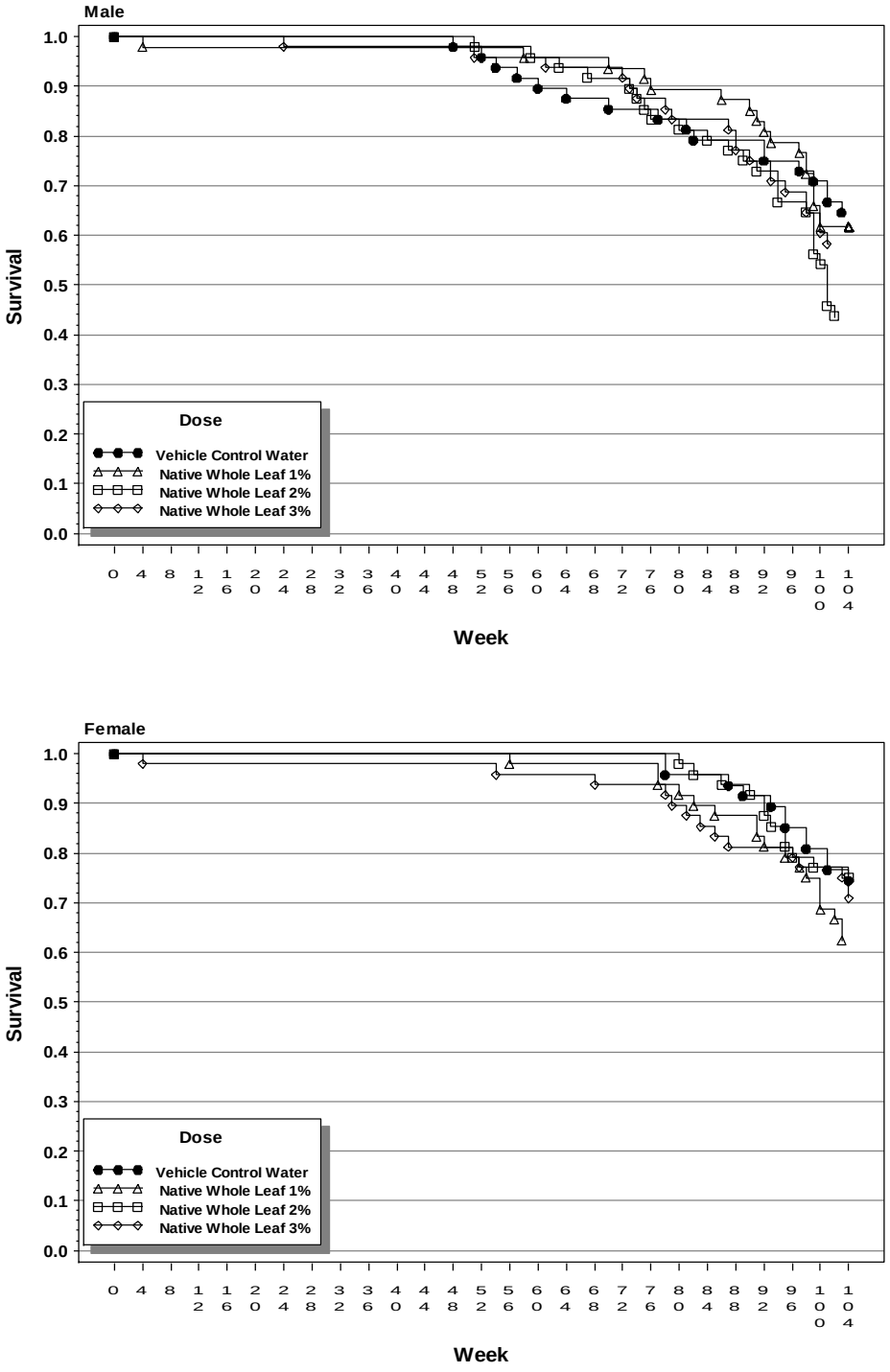


FIGURE 9
Kaplan-Meier Survival Curves for Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

Body Weights and Feed and Water Consumption

The mean body weights of mice throughout the 2-year study are on shown in four week intervals for males in Table 16 and females in Table 17 and are graphically represented in Figure 10. The mean body weights of all groups of exposed male and female mice were within 10% of the controls throughout the study (Table 17 and Figure 10).

With a few exceptions of increased consumption among Aloe vera whole leaf groups, feed consumption patterns were similar across treatment groups in male and female mice.

Mean daily water consumptions among male and female mice are shown in Tables J3 and J4, respectively. Polydipsia was pronounced in male and female mice administered the Aloe vera whole leaf extract, and significant dose-related increases in consumption were observed throughout the study. In pairwise comparison tests with controls, significant increases in water consumption were observed at each dose level of Aloe vera whole leaf in female mice and at the 2.0% and 3.0% dose levels in male mice. Male mice that were administered the 1.0% dose level of Aloe vera whole leaf showed significantly higher intakes of water than control levels beginning at week 16. Compared to controls, increased consumption of water in male and female mice alike continued until the end of the study.

Mean daily water consumptions of the 1.0%, 2.0% and 3.0% Aloe vera whole leaf dose groups of male mice for the 2 year study were 12.03, 14.15, and 15.80 g, respectively, and that of female mice from the same treatment groups were 8.25, 11.74, and 14.08 g, respectively. The amounts of water consumed by the 1.0%, 2.0%, and 3.0% Aloe vera whole leaf dose groups of male mice equated to 154.2%, 181.2%, and 202.4%, respectively, of control levels and to 153.5%, 218.2%, and 261.8%, respectively, of control levels for the same dose groups of female mice (Tables J3 and J4).

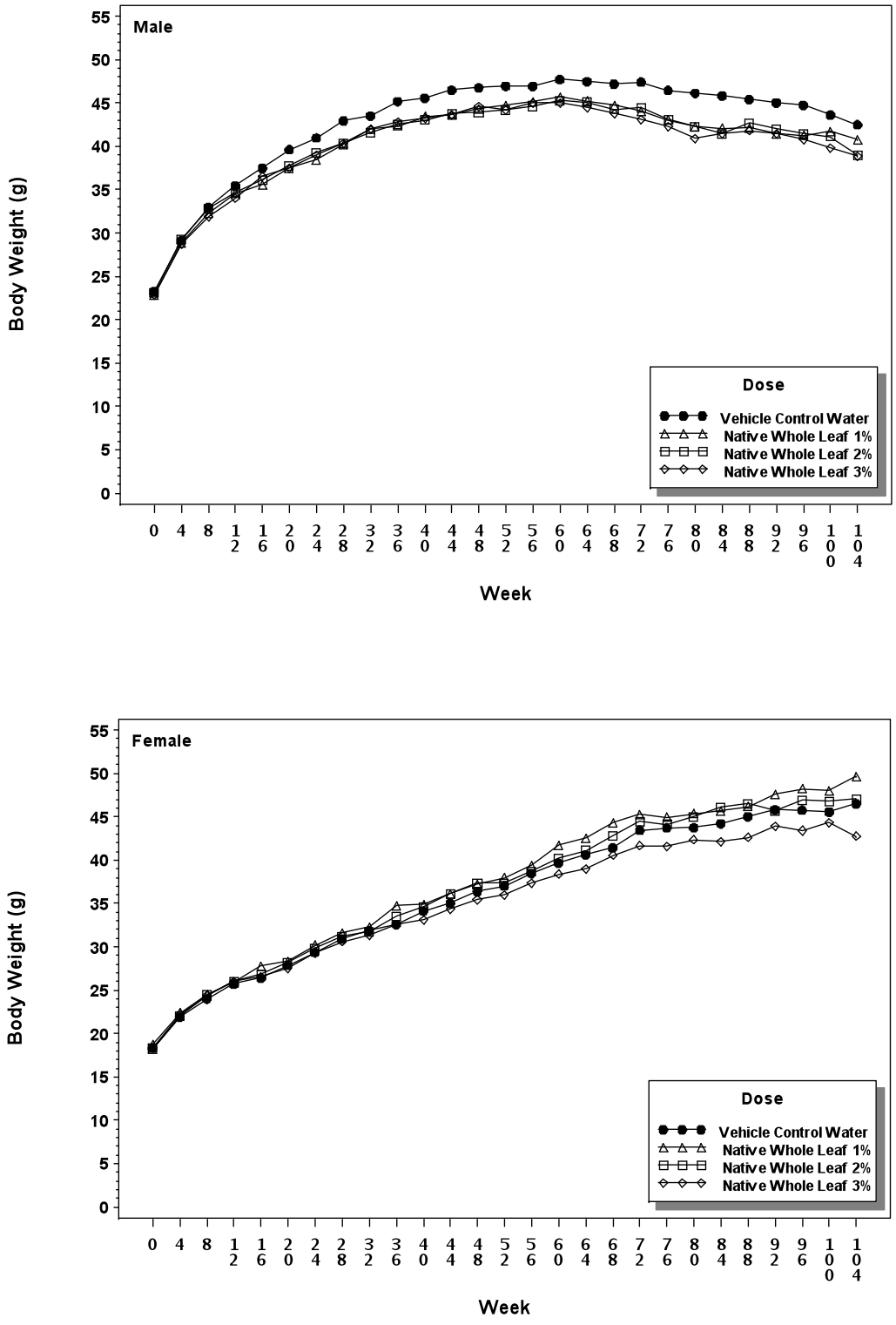


FIGURE 10
Growth Curves for Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

TABLE 16
Mean Body Weights and Survival of Male Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

Weeks on Study	0.0 %		1.0%			2.0%			3.0%		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	29.1	48	28.9	99.2	47	29.3	100.5	48	28.7	98.5	48
8	33.0	48	32.3	97.9	46	32.9	99.7	48	31.9	96.9	48
12	35.4	48	34.6	97.6	46	34.7	98.0	48	34.0	96	48
16	37.5	48	35.6	95.0	46	36.1	96.4	48	36.6	97.6	48
20	39.6	48	37.5	94.7	46	37.8	95.3	48	37.5	94.6	48
24	41.0	48	38.5	93.9	46	39.3	95.8	48	39.0	95.1	48
28	43.0	48	40.3	93.7	46	40.4	94.1	48	40.1	93.4	47
32	43.5	48	42.0	96.5	46	41.6	95.6	48	41.7	95.9	47
36	45.2	48	42.4	93.7	46	42.6	94.2	48	42.6	94.2	47
40	45.6	48	43.5	95.3	46	43.0	94.4	48	43.0	94.2	47
44	46.5	48	43.6	93.9	46	43.8	94.2	48	43.4	93.4	47
48	46.7	48	44.4	94.9	46	43.9	94.0	48	44.4	95.0	47
52	46.8	47	44.8	95.7	46	44.3	94.5	47	44.0	93.9	46
56	47.1	45	45.2	96.0	46	44.7	95.0	47	44.7	95.1	46
60	47.7	44	45.7	95.8	45	45.3	94.8	46	44.7	93.7	46
64	47.5	43	45.3	95.3	45	45.0	94.8	45	44.1	92.8	45
68	47.1	42	44.8	95.0	45	44.2	93.9	44	43.4	92.1	45
72	47.0	41	44.0	93.6	44	44.4	94.5	44	42.7	90.8	45
76	46.1	41	42.9	93.2	43	42.9	93.0	41	41.6	90.1	42
80	45.6	40	42.0	92.1	42	41.6	91.3	40	40.4	88.5	40
84	45.2	38	41.9	92.6	42	40.9	90.4	39	40.9	90.4	40
88	44.9	38	42.0	93.7	41	41.7	92.9	37	41.3	92.1	39
92	44.5	38	41.2	92.6	39	40.9	92.0	35	40.8	91.7	36
96	43.9	36	40.7	92.6	37	40.1	91.4	32	40.0	91.1	33
100	42.7	34	40.3	94.3	31	39.7	93	27	38.9	91.2	31
104	41.5	31	39.1	94.3	29	38.2	92	21	37.6	90.7	27
Mean for weeks											
4-104	43.2		40.9	94.6		40.7	94.3		40.3	93.3	

TABLE 17
Mean Body Weights and Survival of Female Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

Weeks on Study	0.0 %		1.0%			2.0%			3.0%		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	21.9	47	22.4	102.3	48	22.1	100.9	48	22.3	101.7	48
8	24.0	47	24.5	102.1	48	24.4	101.8	48	24.5	101.9	47
12	25.7	47	25.9	100.6	48	26.0	101.2	48	26.1	101.2	47
16	26.4	47	27.8	105.5	48	26.8	101.7	48	26.6	100.8	47
20	27.9	47	28.4	101.8	48	28.2	101.2	48	27.5	98.9	47
24	29.4	47	30.2	102.8	48	29.8	101.5	48	29.3	99.6	47
28	30.9	47	31.6	102.2	48	31.2	101.1	48	30.6	99.0	47
32	31.9	47	32.3	101.5	48	31.8	99.8	48	31.3	98.4	47
36	32.6	47	34.8	106.6	48	33.6	103.0	48	32.6	99.9	47
40	34.1	47	35.0	102.4	48	34.6	101.4	48	33.1	96.9	47
44	35.0	47	36.2	103.3	48	36.1	103.1	48	34.3	98.0	47
48	36.4	47	37.2	102.2	48	37.4	102.7	48	35.5	97.4	47
52	37.0	47	38.0	102.6	48	37.4	101.0	48	36.0	97.1	47
56	38.5	47	39.5	102.5	48	38.8	100.7	48	37.2	96.7	46
60	39.7	47	41.4	104.3	47	40.3	101.5	48	38.3	96.5	46
64	40.6	47	42.2	103.9	47	41.1	101.2	48	38.9	95.7	46
68	41.4	47	44.0	106.2	47	42.9	103.5	48	40.5	97.7	46
72	43.4	47	45.0	103.5	47	44.4	102.3	48	41.7	96.0	45
76	43.7	47	44.7	102.3	47	44.1	100.9	48	41.6	95.2	45
80	43.6	45	45.3	103.9	45	45.1	103.3	48	42.3	96.9	43
84	44.1	45	45.3	102.6	43	45.7	103.7	46	42.5	96.3	41
88	45.2	44	45.9	101.6	42	46.3	102.5	45	42.7	94.4	39
92	45.9	43	46.9	102.4	40	45.7	99.6	44	44.0	95.9	39
96	45.2	40	47.0	104.1	38	46.1	102.0	39	43.4	96.2	39
100	45	38	46.5	103.4	33	45.9	102.0	37	43.7	97.0	37
104	45.5	36	46.4	102	30	46.3	101.9	37	42.2	92.8	36
Mean for weeks											
4-104	36.7		37.9	103.1		37.4	101.8		35.7	97.2	

Drinking water concentrations of 1.0%, 2.0%, or 3.0% (wt/wt) of Aloe vera whole leaf resulted in average daily doses of approximately 2.9, 7.0, or 11.8 g Aloe vera whole leaf/kg of body weight for male mice and 2.2, 6.3, or 11.8 g Aloe vera whole leaf/kg body weight for female mice. The average aloin A and aloe-emodin content of the Aloe vera whole leaf test material was 6.40 and 0.071 mg/g, respectively. The average daily doses of aloin A consumed by the 1.0%, 2.0%, and 3.0% Aloe vera whole leaf groups of mice were 18.8, 44.5, or 75.3 mg aloin/kg body weight for males and 13.9, 40.2, or 75.7 mg aloin/kg body weight for females. The average daily doses of aloe-emodin consumed by the 1.0%, 2.0%, and 3.0% Aloe vera whole leaf groups of mice were 0.2, 0.5, or 0.8 mg aloe-emodin/kg body weight for males and 0.2, 0.4, or 0.8 mg aloe-emodin/kg body weight for females. Aloin A and aloe-emodin were absent in the control water.

Pathology and Histopathology

Complete necropsies were performed on mice that died naturally, were removed from the study as moribund prior to terminal sacrifice, or survived until scheduled terminal sacrifice. The results of pathology examinations were based on 191 male and 191 female mice allocated to the study. Technician errors resulted in the discard of one male mouse from the 1.0% Aloe vera whole leaf dose group and one female mouse from the control group. The results reported for the pathology and histopathology data in the following sections describe the statistically significant or biologically relevant changes in the incidences of neoplasms and nonneoplastic lesions in mice.

Neoplastic Findings

There were no significant increases or decreases in the incidences of neoplastic lesions in male mice. In female mice, a significant dose-dependent decreasing trend in the incidence of anterior pituitary gland adenoma was observed, and significantly lower incidences of this lesion compared to control levels were found in female mice treated with the 2% ($p = 0.046$) and 3% ($p = 0.002$) doses of Aloe vera whole leaf extract.

Nonneoplastic Lesions

Treatment related nonneoplastic lesions appeared primarily in the colon of mice (Tables 18, 19, C3, and D3). In male mice, significant dose dependent increasing trends in the incidences of goblet cell hyperplasia were observed in the ascending, transverse, and descending colon (Table 18). In pairwise comparison tests with same sex controls, treatment related increased incidences of these lesions were observed in male mice at each dose level of Aloe vera whole leaf. In association with goblet cell hyperplasia of the colon, cellular infiltration of the mesenteric lymph

nodes also showed significant dose related increasing trends, and significantly higher cellular infiltration was observed in the mesenteric lymph nodes of the 3.0% Aloe vera whole leaf group of male mice when compared to the control group. Dose-related increasing levels of hyaline droplets (hyaline degeneration) of the nose were also observed in male mice exposed to the Aloe vera whole leaf. The microscopic appearance of the hyaline degeneration of the respiratory epithelium was typical of that seen with the spontaneously occurring hyaline degeneration of the olfactory and respiratory epithelium in $B6C3F_1$ mice, and consisted of accumulation of homogeneous eosinophilic material within the cytoplasm of epithelial cells. Hyaline droplets are considered by pathologists to be a commonly observed non-specific change that occurs in aging mice (Gopinath, 1987). The significance of this lesion is uncertain, but is thought to represent a non-specific adaptive response to the inhalation of irritants.

Similar findings observed in male mice were also found in female mice. Significant dose-related increasing trends in the incidences of goblet cell hyperplasia were observed in the ascending, transverse, and descending colon of female mice. The results of pairwise comparison tests with sex-matched controls showed significantly higher incidences of goblet cell hyperplasia in the ascending and transverse colon at each dose level of Aloe vera whole leaf, and significantly higher incidences than controls were observed in the descending colon of the 2.0% and 3.0% Aloe vera whole leaf groups of female mice (Table 19). Epithelial hyperplasia of the glandular stomach showed a significant dose response to the Aloe vera whole leaf treatment. The significance of this response is uncertain, since significant differences were not observed in comparison tests with the control group and the lesion was not elevated in male mice. Several nonneoplastic lesions were significantly higher in control than treated animals.

TABLE 18
Statistical Analysis of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	1.0%	2.0%	3.0%
Number Necropsied	48	47	48	48
Mesenteric Lymph Node				
Cellular Infiltration				
Overall rate	0/48 (0.0%)	1/45 (2.2%)	4/45 (8.9%)	6/43 (14.0%)
Poly-3 test	P=0.002 **	P=0.49	P=0.053	P=0.012 *
Average Severity	---	3.0	2.3	3.2
Large Intestine				
Ascending Colon Goblet Cell Hyperplasia				
Overall rate[a]	2/47 (4.3%)	16/44 (36.4%)	20/45 (44.4%)	19/42 (45.2%)
Poly-3 test[b]	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity[c]	1.0	1.3	1.6	1.6
Transverse Colon Goblet Cell Hyperplasia				
Overall rate	4/47 (8.5%)	14/44 (31.8%)	21/45 (46.7%)	22/43 (51.2%)
Poly-3 test	P<0.001 ***	P=0.005 **	P<0.001 ***	P<0.001 ***
Average Severity	1.0	1.4	1.7	1.6
Descending Colon Goblet Cell Hyperplasia				
Overall rate	0/47 (0.0%)	7/44 (15.9%)	12/45 (26.7%)	17/43 (39.5%)
Poly-3 test	P<0.001 ***	P=0.006 **	P<0.001 ***	P<0.001 ***
Average Severity	---	1.3	1.5	1.4
All sites examined: Goblet Cell Hyperplasia				
Overall rate	4/47 (8.5%)	17/44 (38.6%)	22/45 (48.9%)	22/44 (50.0%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	1.0	1.4	1.9	1.7
Nose				
Hyaline Droplet				
Overall rate	6/48 (12.5%)	31/47 (66.0%)	39/47 (83.0%)	13/47 (27.7%)
Poly-3 test	P=0.011 *	P<0.001 ***	P<0.001 ***	P=0.054
Average Severity	1.2	1.8	2.0	1.8

[a] Number of lesion-bearing animals/number of animals examined.
[b] P-values under control group column represent results of linear trend tests with increasing dose levels of Aloe vera whole leaf. P-values under exposure group columns represent results of pairwise comparison tests with control groups. P-values that are significant are annotated with an "N" to indicate a negative statistic, "*" to indicate $p < 0.05$, "**" to indicate $p < 0.01$, or "***" to indicate $p<0.001$.
[c] Jonckheere-Terpstra/Shirley-Williams tests for severity scores.

TABLE 19
Statistical Analysis of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	1.0%	2.0%	3.0%
Number Necropsied	47	48	48	48
Glandular Stomach				
Epithelial Hyperplasia				
Overall rate[a]	0/43 (0.0%)	1/44 (2.3%)	3/45 (6.7%)	4/42 (9.5%)
Poly-3 test[b]	P=0.015 *	P=0.488	P=0.124	P=0.052
Average Severity[c]	---	3	2.3	1.8
Large Intestine				
Ascending Colon Goblet Cell Hyperplasia				
Overall rate	1/43 (2.3%)	15/43 (34.9%)	20/44 (45.5%)	25/43 (58.1%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	1.0	1.2	1.3	1.7
Transverse Colon Goblet Cell Hyperplasia				
Overall rate	2/42 (4.8%)	18/42 (42.9%)	23/44 (52.3%)	26/43 (60.5%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	1.0	1.2	1.3	1.7
Descending Colon Goblet Cell Hyperplasia				
Overall rate	0/43 (0.0%)	4/43 (9.3%)	7/44 (15.9%)	17/43 (39.5%)
Poly-3 test	P<0.001 ***	P=0.053	P=0.007 **	P<0.001 ***
Average Severity	---	1.3	1.4	1.6
All sites examined: Goblet Cell Hyperplasia				
Overall rate	3/43 (7.0%)	19/43 (44.2%)	24/44 (54.59%)	28/43 (65.1%)
Poly-3 test	P<0.001 ***	P<0.001 ***	P<0.001 ***	P<0.001 ***
Average Severity	1.0	1.2	1.3	1.7

[a] Number of lesion-bearing animals/number of animals examined.
[b] P-values under control group column represent results of linear trend tests with increasing dose levels of Aloe vera whole leaf. P-values under exposure group columns represent results of pairwise comparison tests with control groups. P-values that are significant are annotated with an "N" to indicate a negative statistic, "*" to indicate $p < 0.05$, "**" to indicate $p < 0.01$, or "***" to indicate $p<0.001$.
[c] Jonckheere-Terpstra/Shirley-Williams tests for severity scores.

DISCUSSION AND CONCLUSIONS

It has been estimated that there are more than 29,000 different nutritional supplements available to consumers, and that Americans spend in excess of 33 billion dollars per year on these supplements (Neuhouser, 2003; Cefalu *et al.*, 2008; Wadman, 2009). Herbal remedies or medicines (a plant or plant part or an extract or mixture of these used to prevent, alleviate, or cure disease) have been used since ancient times as dietary supplements with the intent of prevention or alleviation of specific symptoms of disease (Gibson and Taylor, 2005). Given the fact that herbal products may vary considerably in the content of their purported active ingredients, due to differences in plant growing conditions, processing methods, or to the misidentification of the species of plant used, it is of little surprise that historical or anecdotal information is not a reliable guide to the safety of a given plant.

Aloe vera, a frequently used synonym for the *Aloe barbadensis* Miller plant, has enjoyed a long history of lay acceptance as an herbal remedy and is perhaps the most popular herbal remedy in use today (Klepser *et al.*, 2000; Vogelzang, 2001). Aloe vera has been used traditionally for a variety of purposes, including cosmetic applications, dietary herbal supplementation, prophylaxis therapy, and medicinal treatment for a broad spectrum of illnesses (Marshall, 1990; Klepser *et al.*, 2000; Vogelzang, 2001). In fact, numerous references appear in literature that document the use of Aloe vera for at least 3500 years (Hecht, 1981). However, controversial and contradictory information about this plant abound, with the basis for its use resting mainly on anecdotal information of its therapeutic properties.

Plant material derived from the Aloe plant is characterized by the species of plant (e.g. *Aloe barbadensis*), its source (e.g. the part of the plant), the physical description of the material (e.g. whole leaf extract), and by the constituents (e.g. aloin) found in the material (CIR, 2007). The leaf of the Aloe vera plant consists of two main parts (Klein and Penneys, 1988; Briggs, 1995). One part, the inner central area or leaf pulp, contains large, thin walled cells that produce and store aloe gel, a clear viscous fluid that contains high molecular weight polysaccharides in addition to other constituents. The high molecular weight polysaccharides of the gel are composed mainly of glucose and

mannose sugar moieties joined with β-1→4 glycosidic linkages. Fresh leaf Aloe vera gel contains little to no latex, is not very stable, and deteriorates quickly once the leaf has been damaged or cut (Morgenstern, 2009). The other part of the leaf, the pericyclic tubules, is located beneath the thick rind and within the outer leaf pulp. The pericyclic tubules produce and transport aloe latex along the margins of the leaf. Aloe latex, a yellow bitter exudate, contains anthraquinones and other phenolic substances. In plants, anthraquinones are mostly present as glycosides, where sugar molecules are bound to the anthracene ring by a β-glycosidic linkage (van Gorkom *et al.*, 1999). Many of the anthraquinones are irritants to the gastrointestinal tract and possess cathartic activities (Saito *et al.*, 1989; Teradaira *et al.*, 1993; Atherton, 1998). In its dried form, aloe latex is a drug regulated by the U.S. Food and Drug Administration.

As an herbal remedy, aloe gel can be consumed as is, although pure aloe gel is quite viscous, or added to water or fruit juice that is taken three times per day as a tonic (Williams, 2007). The intact leaves of the Aloe vera plant are utilized to produce aloe whole leaf juice, where just the green parts of the leaf are removed in a process involving cellulase, a cellulose dissolving substance. The resulting extract is yellow in color and retains the content of aloin, the principal anthraquinone in aloe latex. As an herbal remedy, aloe whole leaf juice is promoted for detoxification; it is claimed to cure constipation, help flush out toxins and wastes from the body, promote digestion, and reduce the risk of illnesses (Ayushveda, 2010). Another whole leaf extraction method involves the same process as above, but activated charcoal is added to decolorize and adsorb the anthraquinone components. The resulting aloe whole leaf gel is filtered for clarity to be used as an herbal aloe whole leaf gel drink. Powdered forms of the aloe gel and aloe whole leaf extracts are also used to create capsules that provide 100 to 500 mg per dose. A self-regulating body of Aloe vera producers, the International Aloe Science Council, has been established to certify companies' products according to their standards of quality control. However, even among certified companies, there are differences in the quality and composition of products, due to differences in methods of processing that are used to obtain the Aloe vera products (Morgenstern, 2009). According to the International Aloe Science Council, the maximum allowable aloin content in orally consumed Aloe vera-derived certified materials for non-medical use is 10 ppm (http://www.iasc.org accessed on February 10, 2011).

The National Cancer Institute nominated Aloe vera, as a widely used dietary supplement, for studies by the National Toxicology Program (NTP) because of the potential widespread human exposure to adults, children, infants, and the elderly and because studies suggested that components in Aloe vera may possess tumor-promoting activities.

In the 14-day studies with Aloe vera extracts (Aloe vera gel, Aloe vera decolorized whole leaf, and Aloe vera whole leaf), the doses (0, 0.5%, 1.0%, 1.5%, 2.0%, and 3.0%, wt/wt) were well-tolerated by rats and mice. No treatment-related gross or microscopic lesions were found in rats or mice. Decreased body weight gains and increased relative organ to body weight ratios were observed especially in rats administered the 3% Aloe vera whole leaf extract. The cause of these effects was considered mild to moderate dehydration induced by the cathartic effects of the anthraquinones present in the whole leaf extract. Diarrhea was observed clinically in several groups of rats treated with higher concentrations of Aloe vera extracts. Based on the results of these studies, three doses (0, 1.0%, 2.0%, and 3.0%) were recommended for further toxicity testing in subchronic studies.

In the 13-week studies with Aloe vera whole leaf extract, dose-dependent goblet cell hyperplasia was found in both the cecum and large intestine of rats and mice. In rats, this change was evident in all three dose groups, with the 2.0 and 3.0% dose groups having increased incidence and severity. A similar trend with increasing incidence in goblet cell hyperplasia was observed in mice, although severity was less marked among groups. Rats shared similar findings in males and females, while in mice greater incidence was observed in males. Since administration of the 2.0% Aloe vera whole leaf extract significantly reduced body weight gains in rats, lower doses were recommended for the 2-year bioassays.

In the 2-year mouse study, there were no treatment-related effects on the survival of male and female mice, and changes in body weights were not considered of biological significance. Polydipsia was a pronounced Aloe vera whole leaf dose-related phenomenon observed in male and female mice that resulted in water consumption amounts of greater than 200% relative to controls and average daily doses of approximately 11.8 g Aloe vera whole leaf/kg of body weight for male mice and female mice in the 3.0% dose groups. As found in the 13-week studies, goblet cell hyperplasia of the large intestine was a prominent nonneoplastic lesion in the 2-year mouse study, and treatment-

related increases in the incidences of goblet cell hyperplasia were observed in the colons of male and female mice. There were no treatment-related neoplasms in mice.

Goblet cell hyperplasia and increased mucus production are features of compensatory growth of the intestinal tract after surgical shortening and in ulcerative colitis and Crohn's disease (Ehsanullah *et al.*, 1982; Olubuyide *et al.*, 1984; Kilgore *et al.*, 2000). Moreover, ulcerative colitis and inflammatory bowel disease predispose humans to cancer, and small-bowel resection enhances colorectal carcinogenesis in rats (Prior *et al.*, 1982; Williamson *et al.*, 1982; Sigel *et al.*, 1999). Vaiphei et al. (2004) evaluated biopsies from patients with long-standing (5 or more years) idiopathic ulcerative colitis and correlated incidence of atypical epithelium, goblet cell hyperplasia, and disease duration with the expression of genes known to play roles in cell cycle control and apoptosis. Of the factors evaluated, only goblet cell hyperplasia showed strong correlations with disease duration, p53 gene expression – a tumor suppressor gene that has an important role in cell cycle control and apoptosis, and argyrophilic nucleolar organizer regions (AgNORs) index – a prognostic indicator of early cancer development (Rodrigues *et al.*, 1997). Lee (1988) examined histologically the entire colonic mucosa of 51 human cases of colorectal carcinoma. In 80% of the cases, goblet cell hyperplasia was particularly prominent in the mucosa immediately adjacent to the carcinoma; the mucosa further away from the carcinoma was less frequently (51.0%) affected. The results of these studies suggest that goblet cell hyperplasia may indicate the presence of epithelial cell dysplasia, a precancerous change.

In the 2-year rat study, dose-related decreases in survival were observed in female rats, with survival of the 1.5% Aloe vera whole leaf group considerably less than that of the comparable control group of female rats. A major contributor to early death in this group was the prevalence of cecal dilation, which was the probable cause of death for 12 animals. As observed in the 2-year mouse study, treatment-related increases in water consumption were observed in rats, although the effects on water consumption were less marked in rats. Polydipsia was more evident in males than females; the average daily amount of water consumed by the 3.0% Aloe vera whole leaf dose group of male and female rats was greater than the control group. Mucosal hyperplasia was observed throughout the large intestine of rats, with dose-related increases in incidences and severities. Higher incidences of mucosal hyperplasia were observed in the proximal compared to the distal colon and, in this respect, were similar to the pattern of

incidences of colonic goblet cell hyperplasia observed in the 13-week rat study. These results suggest that, at some point during the course of the study, the exposure of rats to Aloe vera whole leaf induced an insult that caused a progression in the prominence of lesion types from goblet cell hyperplasia to mucosal hyperplasia in the intestinal tract. Whether the observed changes represent one step in a multistep process of carcinogenesis, remains uncertain.

In the 2-year rat study, there were no intestinal neoplasms in control rats. In rats dosed with either 1.0% or 1.5% Aloe vera whole leaf extract, there was a significant increase in neoplasms of the large intestine treatment-related neoplasms were limited to the colon and occurred as adenomas and carcinomas in the ileo-cecal junction (proximal colon, cecum, and ascending, and transverse colon) of both male and female rats. There was no treatment related increase in incidence of neoplasms in any other tissue in either male or female rats. The carcinogenic response to Aloe vera whole leaf administration was greater in males than females and in groups that received the higher dose levels (1.0% and 1.5%) of the Aloe vera whole leaf extract. Higher incidences of neoplastic lesions occurred in the proximal sites of the large intestine than in more distal sections, with no neoplasms occurring in the rectum of either sex. There were no incidences of adenomas or carcinomas in the 0.5% Aloe vera whole leaf groups. The age-adjusted incidences of adenomas or carcinomas were 21% and 39% in the 1.0% and 1.5% Aloe vera whole leaf female groups, respectively, and 67% and 74% in the 1.0% and 1.5% Aloe vera whole leaf male groups, respectively. Neoplasms in the rat were confined within the mucosal wall of the large intestine and did not metastasize to regional mesenteric lymph nodes or more distant sites.

Because rodents do not usually develop spontaneous neoplasms of the colon, carcinogen induction of neoplasms in rodent colons has been used as a model for human colon cancer development and for evaluating chemopreventive regimens (Holt *et al.*, 1996; Tanaka, 2009). The most commonly induced tumors of the rodent gastrointestinal tract are squamous neoplasms of the forestomach in mice and intestinal carcinoma of the colon in rats (Chandra *et al.*, 2009).

The Aloe vera whole leaf extract used in these studies contained the components of the Aloe vera gel and Aloe vera latex. Many of the polysaccharides in aloe gel are polydispersed β–(1→4)-linked glucomannans, mannans, or pectins of a wide range of molecular weights and interspersed with O-acetyl groups (Tanaka *et al.*, 2006). Due to

the β-glycosidic linkage between sugar moieties (primarily mannose and glucose), many of these non-starch polysaccharides, like cellulose, are resistant to hydrolysis by acid in the stomach and α-glucosidase activity in the small intestine and reach the colon as undigested fiber, where they may be fermented by the colonic microflora.

Most small herbivorous birds and some omnivorous mammals, such as the rat and other rodents, compensate for a limited gut capacity and higher rate of metabolism by selective retention of fluid and small particles in their cecum (Johnson *et al.*, 1960). The cecum, a large pouch or tube-like structure, is considered the first part of the large intestine. In humans, the cecum functions to absorb fluids and salts and to mix its contents with mucus (MacFie, 2004). Rodents and other herbivorous monogastric species of vertebrates, such as rabbits and horses, obtain a substantial portion of their nutrients by the retention and microbial fermentation of plant material in the cecum. In this respect, the cecum is somewhat analogous to the bovine rumen and serves as an important organ of absorption (Stevens and Hume, 1998). Dietary components incompletely digested and/or absorbed in the small intestine, such as cellulose, give rise to an increased amount of osmotically active material in the intestinal contents. The amount of osmotically active material may increase further in the cecum, if the non-absorbed material can be utilized as a substrate by the cecal bacteria and if bacterial metabolism results in the production of low-molecular-weight metabolites that are not readily absorbed. The increase in the amount of osmotically active material results in an increase in water retention, so the animals tend to drink more fluid, and the cecum distends in size due to the increase in contents. The muscular contractions of the cecum pass digested and incompletely digested dietary bulk to the next region of the large intestine, the proximal colon. Evidence suggests that the rate at which fluid and small particles leave the cecum is determined by the rate of cecal fermentation and the degree of fluid distention (Stevens and Hume, 1998). Cecal dilatation (enlargement) is a physiological adaptation process that in itself is of no toxicological significance; however, side-effects of cecal impaction and severe diarrhea have detrimental effects on the health of animals (Johnson *et al.*, 1960; Jain *et al.*, 2007). The recommended amount of cellulose, as bulk, for optimal growth in rats is 2%; rats died when dietary cellulose was 60% (Yang *et al.*, 1969; van Zyl *et al.*, 1999).

Dilatation of the rodent cecum, often accompanied with severe diarrhea, has been reported for a variety of compounds (Leegwater *et al.*, 1974; Birnbaum *et al.*, 1986; Til *et al.*, 1986; Smits-Van Prooije *et al.*, 1990; Courtney, 2000). The activities of the rat cecum in the digestion of the Aloe vera whole leaf carbohydrates in this

study likely mimic that in the digestion of cellulose, although studies were not conducted to determine the accuracy of this statement. Polydipsia, diarrhea, and cecal dilatation were common observances in this study, and cecal dilatation was the noted cause of death for a number of rats. Cecal dilatation may have resulted from an accumulation of undigested Aloe vera whole leaf carbohydrates or volatile gases produced by microbiota in their efforts to digest the β-glycosidic linked carbohydrates. In order to relieve the enlarged cecum of its contents, animals then consumed more water. However, the water contained the very same materials that caused the dilatation. Cecal impaction and diarrhea was observed in rats in the 2-year study. Cecal dilatation and cecal impaction were not observed by Ikeno et al (2002) during the life-long administration to F344 rats of whole leaf decolorized aloe gel (0.02%) in the drinking water or when the rats were fed diets containing 1.0% Aloe vera crude gel or 1.0% whole leaf decolorized aloe gel. In the production of decolorized aloe gel, the charcoal is used to remove anthraquinone components of aloe latex; however, the process also removes some of the high molecular weight polysaccharides of the inner leaf aloe gel (Waller *et al.*, 2004). The lower concentration of aloe materials and the loss of some of the polysaccharides may have masked the effects observed in the present study. Additionally, the influence of dietary composition on the absorption and metabolism of Aloe vera has been demonstrated (Koch, 1996).

In plants, the majority of anthraquinones appear as anthraquinone O-glycosides, dianthrone O-glycosides, or, as in the case of Aloe vera, anthraquinone C-glycosides. Due to the β-glycosidic linkage between the sugar and the anthracene ring structure and the hydrophilic nature of the molecules, the anthraquinone C-glycosides in aloe latex are protected, after oral administration, from acid hydrolysis in the stomach and enzymatic activity in small intestine and are carried unabsorbed to the large intestine of rats, where *Eubacterium* sp. act upon the C-glycoside anthranoids to release glucose and the free aglycone (Hattori *et al.*, 1993; van Gorkom *et al.*, 1999). Studies have shown that the cathartic effects of the aloe latex are not due to the ingested form of the anthraquinone, aloin, but rather to the aglycone, aloe-emodin-9-anthrone, formed by bacterial metabolism of the aloin parent compound (Akao *et al.*, 1996). The *Eubacterium* sp is expressed differentially across mammalian species; therefore, not all mammalian species are capable of transforming aloin to the aloe-emodin anthrone (Werner, 2007; Canny and McCormick, 2008). In humans, the transformation of aloin to the purgative component, aloe-emodin anthrone, is

carried out by the intestinal anaerobe, *Eubacterium* sp. strain BAR (Che *et al.*, 1991; Hattori *et al.*, 1993; Akao *et al.*, 1996).

Studies have shown that the cecum is the site for bacterial metabolism of anthraquinones in the rat large intestine and that aloe-emodin anthrone is formed in cecal contents (Dreessen *et al.*, 1981; Dreessen and Lemli, 1988). However, Akao *et al*. (1996) did not find diarrhea in male Wistar rats orally administered aloin (100 mg/kg) via gavage, in spite of observing severe diarrhea with sennoside B (40 mg/kg), an anthraquinone of the senna plant. The potency of aloin to exert cathartic activities is known to vary among animal species, e.g. aloin shows purgative potency in humans but has little activity in the rat and mouse (Hattori *et al.*, 1988; Che *et al.*, 1991; Joshi, 1998). Severe diarrhea was induced approximately 7 h after the oral administration of barbaloin via gavage to gnotobiotic rats mono-associated with *Eubacterium* sp. strain BAR (Akao *et al.*, 1996). Therefore, whether aloin is metabolized to its purgative principle in amounts sufficient to induce catharsis by microflora in the rat large intestine is uncertain.

Soft and mud-like feces and neoplasms in rat colons were observed in the 2-year study, suggesting that the presence of Aloe vera latex in the drinking water may be a causative cathartic and/or carcinogenic factor in rats. Similar findings were observed in rats that received dietary administration of *Aloe Arborescens* Miller, a species of Aloe used commercially as a food additive. Shimpo *et al*. (2001) studied the modifying effects of freeze-dried whole leaf *Aloe arborescens* Miller var. *natalensis* Berger (ALOE) on azoxymethane-induced intestinal carcinogenesis in rats that were fed diets containing 1.0% or 5.0% ALOE for 5 weeks. The 5% ALOE dose level decreased body weights and induced soft feces in rats; however, both 1.0% and 5% ALOE-enriched diets were found to offer protection from azoymethane-induced intestinal carcinogenesis.

Aloe arborescens Miller, different species and variety of Aloe, was used for assessment of toxicity and carcinogenic potential in one and two year studies in rats (Matsuda *et al.*, 2008; Yokohira *et al.*, 2009). In the 1-year study, the *Aloe arborescens* was added to the basal diet of Wistar rats at 0, 0.16%, 0.8%, or 4.0%, and both male and female rats showed diarrhea, reduced body weight gains, and severe sinus dilatation of the ileocecal lymph nodes (Matsuda *et al.*, 2008). In 2-year studies, rats received *Aloe arborescens* at concentrations of 0, 0.8%, or 4.0% in the diet (Yokohira *et al.*, 2009). Diarrhea or loose stools, decreased body weights, severe sinus dilatation in the ileocecal

lymph, and adenomas and adenocarcinomas of the large intestine developed in the 4% high dose group of rats. Adenocarcinomas were observed in the cecum and colon with an incidence of 2% in male rats, and adenomas occurred in the colon with an incidence of approximately 7% in male and female rats and with an incidence of 2% in the rectum of male rats. Incidences of tumors in the cecum, colon, and rectum combined were significantly elevated in 4% males. The effects and lesions found in the 2-year study of *Aloe arborescens* Miller were remarkably similar, albeit to a much lesser degree of severity, as those found in the present 2-year study on Aloe vera. It is known that purgative effects of aloe are influenced by dietary components, since the metabolism of aloin to aloe-emodin is promoted by a diet that contains iron salts and iron-rich meat and decreased by cereals and complex carbohydrates (Koch, 1996). *Aloe arborescens* Miller is used commercially as an industrial food additive; however, as with Aloe vera, *Aloe arborescens* Miller has been long used as an herbal remedy for gastrointestinal complaints, skin injuries, and burns (Matsuda *et al.*, 2008).

The associations between colorectal cancer risk and anthraquinone laxative use remain controversial (Nascimbeni *et al.*, 2002; Willems *et al.*, 2003). It has been well documented that 1,8-dihydroxyanthraquinone (danthrone) exerts tumor-promoting effects and tumorigenic activities in the large intestine of rodents (Mori *et al.*, 1985; Mori *et al.*, 1990). Furthermore, Nishikawa *et al*. (1997) showed that danthrone enhanced epithelial mucosal cell replication in the large intestine, especially in the cecum of rats, and was associated with elevated prostaglandin E_2 levels that correlated with dose of danthrone and the severity of diarrhea. Paradoxically, anti-tumorigenic effects are equally well documented for some anthraquinones (Grimaudo *et al.*, 1997; Zhao *et al.*, 1999; El-Shemy *et al.*, 2010). A purified senna extract did not show any carcinogenic potential when administered via the drinking water at daily doses of 0, 5, 15, and 25 mg/kg body weight to male and female rats for 2 years (Lyden-Sokolowski *et al.*, 1993). A laxative effect in high-dose females and in mid- and high-dose males and treatment-related mesenteric lymph node hyperplasia were observed, but no differences in the incidences of neoplasms were found between control and high-dose animals.

Increased incidences of mesenteric lymph node hyperplasia and degeneration were also observed in rats exposed to the Aloe vera whole leaf, suggesting that the Aloe vera whole leaf might induce an immune response. In normal

rodents, lymph node hyperplasia may be found to varying degrees depending upon the age of the animal, its health status, the location of the lymph node, or even the plane of the lymph node section (Elmore, 2006b). This is of particular importance for mesenteric lymph nodes, which may show a wide variation in degree of hyperplasia between animals due to stimulation by antigens in the intestinal tract (Elmore, 2006a). Lymph node hyperplasia is generally a reactive or immune response and is not considered a pre-neoplastic lesion (Cesta, 2006).

Anthranoid laxatives as a component of herbal remedies, such as Aloe vera, are commonly used as a self-medication for chronic constipation. At present, the available reports seem to suggest that the anthraquinones induce cell proliferation in the large intestine due to irritation of the mucosal lining and heightened immune responses. The irritation may be induced via effects on the microflora population and alterations in the production of short-chain fatty acids (Pogribna *et al.*, 2008).

The whole leaf extract of the Aloe vera plant contains aloe gel from the inner leaf pulp and aloe latex from the leaf pericyclic tubules. Both of these components may have played a role in the development of colon cancer in rats in this 2-year study. The constituents of aloe gel and aloe latex are each composed of β-linked glycosyl residues that, like cellulose, reach the large intestine in mostly undigested form. Rats have a large cecum that serves as the principle site of microbial fermentation, and the microflora in the rat cecum have been shown capable of metabolizing β-linked glycosyl residues.

It has been postulated that the size of the rat cecum is controlled by the osmotic value of the cecal contents. Incompletely digested and undigested food substances give rise to an increased amount of osmotically active material in the cecal contents and results in an increase in water retention, so the animals tend to drink more fluid. Cecal dilatation with possible progression to impaction, and severe diarrhea often accompany these events. Cecal dilatation (enlargement) and impaction, significantly increased water intake, and diarrhea were observed in rats on the 2-year study.

There is some debate in the literature as to whether the fermentability of a fiber plays a role in its protection or promotion of colon carcinogenesis (Lupton, 2004; Hamer *et al.*, 2008). Additionally, there is a strong association

with a possibly increased risk of colon carcinoma and anthranoid self-medication for constipation and purgative purposes.

Future investigations of Aloe vera should focus on the effects of the β-linked complex polysaccharides of the Aloe vera gel in the functioning rat gastrointestinal tract, in particular the cecum, and the independent and synergistic effects contributed by the anthraquinones of the Aloe vera latex to the carcinogenic effects observed in this study.

CONCLUSIONS See corrected text below:

~~Under the conditions of these 2-year studies, *there was clear evidence of carcinogenic activity* of an Aloe vera (*Aloe barbadensis* Miller) whole leaf extract in male or female F344/N rats based upon increased incidences of neoplasms (adenomas and carcinomas) on the large intestine. *There was no evidence of carcinogenic activity* of the whole leaf extract of Aloe vera in male or female B6C3F$_1$ mice exposed to 1.0%, 2.0%, or 3.0 % (wt/wt) Aloe vera whole leaf extract in drinking water.~~

Corrected text:

Under the conditions of these 2-year drinking water studies, there was *clear evidence of carcinogenic activity* of a nondecolorized whole leaf extract of Aloe vera in male and female F344/N rats based upon increased incidences of adenomas and carcinomas of the large intestine. There was *no evidence of carcinogenic activity* in male or female B6C3F$_1$ mice exposed to 1.0%, 2.0%, or 3.0% (wt/wt) Aloe vera whole leaf extract in drinking water.

Exposure to a nondecolorized whole leaf extract of Aloe vera resulted in increased incidences of nonneoplastic lesions of the large intestine in male and female rats and mice, the small intestine of male and female rats, the stomach in male and female rats and female mice, the mesenteric lymph nodes in male and female rats and male mice, and the nose in male mice.

REFERENCES

Abebe, W. (2003). An overview of herbal supplement utilization with particular emphasis on possible interactions with dental drugs and oral manifestations. *J Dent Hyg* **77**, 37-46.

Ajabnoor, M. A. (1990). Effect of aloes on blood glucose levels in normal and alloxan diabetic mice. *J Ethnopharmacol* **28**, 215-220.

Akao, T., Che, Q. M., Kobashi, K., Hattori, M., and Namba, T. (1996). A purgative action of barbaloin is induced by Eubacterium sp. strain BAR, a human intestinal anaerobe, capable of transforming barbaloin to aloe-emodin anthrone. *Biol Pharm Bull* **19**, 136-138.

Akev, N., and Can, A. (1999). Separation and some properties of Aloe vera L. leaf pulp lectins. *Phytother Res* **13**, 489-493.

Alves, D. S., Perez-Fons, L., Estepa, A., and Micol, V. (2004). Membrane-related effects underlying the biological activity of the anthraquinones emodin and barbaloin. *Biochem Pharmacol* **68**, 549-561.

Arosio, B., Gagliano, N., Fusaro, L. M., Parmeggiani, L., Tagliabue, J., Galetti, P., De Castri, D., Moscheni, C., and Annoni, G. (2000). Aloe-Emodin quinone pretreatment reduces acute liver injury induced by carbon tetrachloride. *Pharmacol Toxicol* **87**, 229-233.

Atherton, P. (1998). Aloe vera: magic or medicine? *Nurs Stand* **12**, 49-52, 54.

Ayushveda (2010). Aloe drink - Benefits of Aloe vera juice. http://www.us.ayushveda.com/aloe-dring-benefits-of-aloe-vera-juice/.

Bailer, A. J., and Portier, C. J. (1988). Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* **44**, 417-431.

Bieler, G. S., and Williams, R. L. (1993). Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics* **49**, 793-801.

Birch, A. J., and Donovan, F. W. (1955). Barbaloin. I. Some observations on its structure. *Australian Journal of Chemistry* **8**, 523-528.

Birnbaum, L. S., Deskin, R., Grumbein, S. L., Kurtz, P., Fowler, K. L., and Peters, A. C. (1986). Prechronic toxicity of o-benzyl-p-chlorophenol in rats and mice. *Fundam Appl Toxicol* **7**, 615-625.

Bischoff, J. (1995). Approaches to studying cell adhesion molecules in angiogenesis. *Trends Cell Biol* **5**, 69-74.

Bouthet, C. F., Schirf, V. R., and Winters, W. D. (1995). Stimulation of neuron-like cell growth by aloe substances. *Phytother Res* **9**, 185-188.

Bowles, W. B. (1994). Aloe vera gel and its effect on cell growth. *Parfumerie und Kosmetik* **75**, 660-661.

Breier, G., and Risau, W. (1996). The role of vascular endothelial growth factor in blood vessel formation. *Trends in Cell Biology* **6**, 454-456.

Briggs, C. (1995). Herbal medicine:aloe. *Canadian Pharmaceutical Journal* **128**, 48-50.

Brusick, D., and Mengs, U. (1997). Assessment of the genotoxic risk from laxative senna products. *Environ Mol Mutagen* **29**, 1-9.

Bunyapraphatsara, N., Yongchaiyudha, S., Rungpitarangsi, V., and Chokechaijaroenporn, N. (1996). Antidiabetic activity of aloe vera L juice. II. clinical trail in diabetes mellitus patients in combination with glibenclamide. *Phytomedicine* **3**, 245-248.

Canny, G. O., and McCormick, B. A. (2008). Bacteria in the intestine, helpful residents or enemies from within? *Infect Immun* **76**, 3360-3373.

Capasso, F., Mascolo, N., Autore, G., and Duraccio, M. R. (1983). Effect of indomethacin on aloin and 1,8 dioxianthraquinone-induced production of prostaglandins in rat isolated colon. *Prostaglandins* **26**, 557-562.

Cefalu, W. T., Ye, J., and Wang, Z. Q. (2008). Efficacy of dietary supplementation with botanicals on carbohydrate metabolism in humans. *Endocr Metab Immune Disord Drug Targets* **8**, 78-81.

Cesta, M. F. (2006). Normal structure, function, and histology of mucosa-associated lymphoid tissue. *Toxicol Pathol* **34**, 599-608.

Chandra, S. A., Nolan, M. W., and Malarkey, D. E. (2009). Chemical carcinogenesis of the gastrointestinal tract in rodents: an overview with emphasis on NTP carcinogenesis bioassays. *Toxicol Pathol* **38**, 188-197.

Chauser-Volfson, E., and Gutterman, Y. (1996). The barbaloin content and distribution in *Aloe arborescens* leaves according to the leaf part, age, position, and season. *Israel Journal of Plant Science* **44**, 289-296.

Che, Q. M., Akao, T., Hattori, M., Kobashi, K., and Namba, T. (1991). Isolation of a human intestinal bacterium capable of transforming barbaloin to aloe-emodin anthrone. *Planta Med* **57**, 15-19.

Chen, H. C., Hsieh, W. T., Chang, W. C., and Chung, J. G. (2004). Aloe-emodin induced in vitro G2/M arrest of cell cycle in human promyelocytic leukemia HL-60 cells. *Food Chem Toxicol* **42**, 1251-1257.

Choi, S., Kim, K. W., Choi, J. S., Han, S. T., Park, Y. I., Lee, S. K., Kim, J. S., and Chung, M. H. (2002). Angiogenic activity of beta-sitosterol in the ischaemia/reperfusion-damaged brain of Mongolian gerbil. *Planta Med* **68**, 330-335.

Choi, S. W., Son, B. W., Son, Y. S., Park, Y. I., Lee, S. K., and Chung, M. H. (2001). The wound-healing effect of a glycoprotein fraction isolated from aloe vera. *Br J Dermatol* **145**, 535-545.

Chow, J. T.-N., Williamson, D. A., Yates, K. M., and Goux, W. J. (2005). Chemical characterization of the immunomodulating polysaccharide of *Aloe vera* L. *Carbohydrate Research* **340**, 1131-1142.

CIR (2007). Final report of the on the safety assessment of AloeAndongensis Extract, Aloe Andongensis Leaf Juice,aloe Arborescens Leaf Extract, Aloe Arborescens Leaf Juice, Aloe Arborescens Leaf Protoplasts, Aloe Barbadensis Flower Extract, Aloe Barbadensis Leaf, Aloe Barbadensis Leaf Extract, Aloe Barbadensis Leaf Juice,aloe Barbadensis Leaf Polysaccharides, Aloe Barbadensis Leaf Water, Aloe Ferox Leaf Extract, Aloe Ferox Leaf Juice, and Aloe Ferox Leaf Juice Extract. *Int J Toxicol* **26 Suppl 2**, 1-50.

Cooke, W. T. (1981). Laxative abuse. *Acta Gastroenterol Belg* **44**, 448-458.

Courtney, C. L. (2000). Cecal torsion in rodents associated with chronic administration of clinafloxacin. *Toxicol Pathol* **28**, 643-648.

Danhof, I. E. (1998). Position statement on polysaccharides.

Danhof, I. E., and McAnally, B. H. (1983). Stabilized aloe vera: Effect on human skin cells. *Drug Cosmet Ind* **133**, 52-106.

Davis, R. H., Agnew, P. S., and Shapiro, E. (1986). Antiarthritic activity of anthraquinones found in aloe for podiatric medicine. *J Am Podiatr Med Assoc* **76**, 61-66.

Davis, R. H., Donato, J. J., Hartman, G. M., and Haas, R. C. (1994). Anti-inflammatory and wound healing activity of a growth substance in Aloe vera. *J Am Podiatr Med Assoc* **84**, 77-81.

Davis, R. H., Kabbani, J. M., and Maro, N. P. (1987a). Aloe vera and wound healing. *J Am Podiatr Med Assoc* **77**, 165-169.

Davis, R. H., Leitner, M. G., and Russo, J. M. (1987b). Topical anti-inflammatory activity of Aloe vera as measured by ear swelling. *J Am Podiatr Med Assoc* **77**, 610-612.

Davis, R. H., Leitner, M. G., and Russo, J. M. (1988). Aloe vera. A natural approach for treating wounds, edema, and pain in diabetes. *J Am Podiatr Med Assoc* **78**, 60-68.

Davis, R. H., Stewart, G. J., and Bregman, P. J. (1992). Aloe vera and the inflamed synovial pouch model. *J Am Podiatr Med Assoc* **82**, 140-148.

deWitte, P. (1993). Metabolism and pharmacokinetics of anthranoids. *Pharmacology* **47**, 86-97.

deWitte, P., and Lemli, L. (1990). The metabolism of anthranoid laxatives. *Hepatogastroenterol* **37**, 601-605.

Dreessen, M., Eyssen, H., and Lemli, J. (1981). The metabolism of sennosides A and B by the intestinal microflora: in vitro and in vivo studies on the rat and the mouse. *J Pharm Pharmacol* **33**, 679-681.

Dreessen, M., and Lemli, J. (1988). Studies in the field of drugs containing anthraquinone derivatives. XXXVI. The metabolism of cascarosides by intestinal bacteria. *Pharm Acta Helv* **63**, 287-289.

Duke, J. A., and Beckstrom-Sternberg, S. M. (1994). "Acceptable" Levels of Flavoring Ingredients? *Developmental Food Science* **34**, 741-757.

Dykman, K. D., Tone, C., Ford, C., and Dykman, R. A. (1998). The effects of nutritional supplements on the symptoms of fibromyalgia and chronic fatigue syndrome. *Integr Physiol Behav Sci* **33**, 61-71.

Ehsanullah, M., Filipe, M. I., and Gazzard, B. (1982). Mucin secretion in inflammatory bowel disease: correlation with disease activity and dysplasia. *Gut* **23**, 485-489.

El-Shemy, H. A., Aboul-Soud, M. A., Nassr-Allah, A. A., Aboul-Enein, K. M., Kabash, A., and Yagi, A. (2010). Antitumor properties and modulation of antioxidant enzymes' activity by Aloe vera leaf active principles isolated via supercritical carbon dioxide extraction. *Curr Med Chem* **17**, 129-138.

Elmore, S. A. (2006a). Enhanced histopathology of mucosa-associated lymphoid tissue. *Toxicol Pathol* **34**, 687-696.

Elmore, S. A. (2006b). Histopathology of the lymph nodes. *Toxicol Pathol* **34**, 425-454.

ElSohly, M.A. and Gul, W. (2007). Determination of the anthraquinones aloe-emodin and aloin-A by liquid chromatography with mass spectrometric and diode array detection. J of AOAC Intn **90**, 28-42.

Eshun, K., and He, Q. (2004). Aloe vera: a valuable ingredient for the food, pharmaceutical and cosmetic industries--a review. *Crit Rev Food Sci Nutr* **44**, 91-96.

Evangelos, C., Spyros, K., and Spyros, D. (2005). Henoch-Schonlein purpura associated with Aloe vera administration. *Eur J Intern Med* **16**, 59-60.

Fantus, B. (1922). Aloes as medicine. *Journal of the American Pharmaceutical Association* **XI**, 616-621.

Femenia, A., Sanchez, E. S., Simal, S., and Rossello, C. (1999). Compositional features of polysacchardies from Aloe vera (Aloe barbadensis Miller) plant tissues. *Carbohydrate Polymers* **39**, 109-117.

Ferro, V. A., Bradbury, F., Cameron, P., Shakir, E., Rahman, S. R., and Stimson, W. H. (2003). In vitro susceptibilities of Shigella flexneri and Streptococcus pyogenes to inner gel of Aloe barbadensis Miller. *Antimicrob Agents Chemother* **47**, 1137-1139.

Fogleman, R. W., Chapdelaine, J. M., Carpenter, R. H., and McAnalley, B. H. (1992a). Toxicologic evaluation of injectable acemannan in the mouse, rat and dog. *Vet Hum Toxicol* **34**, 201-205.

Fogleman, R. W., Shellenberger, T. E., Balmer, M. F., Carpenter, R. H., and McAnalley, B. H. (1992b). Subchronic oral administration of acemannan in the rat and dog. *Vet Hum Toxicol* **34**, 144-147.

Folkman, J., and Klagsbrun, M. (1987). Angiogenic factors. *Science* **235**, 442-447.

Franz, G., and Grun, M. (1983). Chemistry, occurence and biosynthesis of C-glycosyl compounds in plants. *Planta Med* **47**, 131-140.

Ganet, W. B. M., and van Schooten, C. A. M. (1992). Water requirement of Aloe vera in a dry caribbean climate. *Irrigation Science* **13**, 81-85.

Ghannam, N., Kingston, M., Al-Meshaal, I. A., Tariq, M., Parman, N. S., and Woodhouse, N. (1986). The antidiabetic activity of aloes: preliminary clinical and experimental observations. *Horm Res* **24**, 288-294.

Gibson, J. E., and Taylor, D. A. (2005). Can claims, misleading information, and manufacturing issues regarding dietary supplements be improved in the United States? *J Pharmacol Exp Ther* **314**, 939-944.

Gopinath, C. (1987). *Atlas of experimental toxicological pathology*. MTP Press, Lancaster.

Gorloff, D. R. (1983). Study of the organoleptic properties of the exuded mucilage from aloe barbadensis leaves. *Erde International* **1**, 46-59.

Gowda, D. C., Neelisiddaiah, B., and Anjaneyalu, Y. V. (1979). Structural studies of polysaccharides from *Aloe vera*. *Carbohydrate Research* **72**, 201-205.

Gramatica, P., Monti, D., Speranza, G., and Manitto, P. (1982). Aloe revisited. The structure of Aloeresin A. *Tetrahedron Letters* **23**, 2423-2424.

Grimaudo, S., Tolomeo, M., Gancitano, R. A., D'Alessandro, N., and Aiello, E. (1997). Effects of highly purified anthraquinoid compounds from Aloe vera on sensitive and multidrug resistant leukemia cells. *Oncology Reports* **4**, 341-343.

Grindlay, D., and Reynolds, T. (1986). The Aloe vera phenomenon: a review of the properties and modern uses of the leaf parenchyma gel. *J Ethnopharmacol* **16**, 117-151.

Groom, Q. J., and Reynolds, T. (1986). Barbaloin in Aloe species. *Planta Med.* **52**, 345-348.

Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J., and Brummer, R. J. (2008). Review article: the role of butyrate on colonic function. *Aliment Pharmacol Ther* **27**, 104-119.

Hanley, D. C., Solomon, W. A., Saffran, B., and Davis, R. H. (1982). The evaluation of natural substances in the treatment of adjuvant arthritis. *J Am Podiatry Assoc* **72**, 275-284.

Hattori, M., Akao, T., Kobashi, K., and Namba, T. (1993). Cleavages of the O- and C-glucosyl bonds of anthrone and 10,10'-bianthrone derivatives by human intestinal bacteria. *Pharmacology* **47 Suppl 1**, 125-133.

Hattori, M., Kanda, T., Shu, Y. Z., Akao, T., Kobashi, K., and Namba, T. (1988). Metabolism of barbaloin by intestinal bacteria. *Chem Pharm Bull (Tokyo)* **36**, 4462-4466.

Hay, J. E., and Haynes, L. J. (1956). The aloins. Part I. The structure of Barbaloin. *Journal of the Chemical Society*, 3141-3147.

Hayes, S. M. (1999). Lichen planus--report of successful treatment with aloe vera. *Gen Dent* **47**, 268-272.

Haynes, L. J., and Holdsworth, D. K. (1970). *C*-glycosyl compounds. Part VI. Aloesin, a *C*-glucosylchromone from *Aloe* sp. *Journal of the Chemical Society (C)* **18**, 2581-2586.

Hecht, A. (1981). The overselling of aloe vera. *FDA Consumer* **15**, 26-29.

Heizer, W. D., Warshaw, A. L., Waldmann, T. A., and Laster, L. (1968). Protein-losing gastroenteropathy and malabsorption associated with factitious diarrhea. *Ann Intern Med* **68**, 839-852.

Herlihy, J. T., Bertrand, H. A., Kim, J. D., Ikeno, Y., and Yu, B. P. (1998a). Effects of aloe vera ingestion in the rat I. Growth, food and fluid intake and serum chemistry. *Phytother Res* **12**, 183-188.

Herlihy, J. T., Kim, J. D., Katu, D. N., Nelson, J. F., Ward, W. F., Ikeno, Y., and Yu, B. P. (1998b). Effects of aloe vera ingestion in the rat. II. Hormonal and metabolic characteristics. *Phytother Res* **12**, 355-360.

Holt, P. R., Mokuolu, A. O., Distler, P., Liu, T., and Reddy, B. S. (1996). Regional distribution of carcinogen-induced colonic neoplasia in the rat. *Nutr Cancer* **25**, 129-135.

Hutter, J. A., Salman, M., Stavinoha, W. B., Satsangi, N., Williams, R. F., Streeper, R. T., and Weintraub, S. T. (1996). Antiinflammatory C-glucosyl chromone from Aloe barbadensis. *J Nat Prod* **59**, 541-543.

Ikeno, Y., Hubbard, G. B., Lee, S., Yu, B. P., and Herlihy, J. T. (2002). The influence of long-term Aloe vera ingestion on age-related disease in male Fischer 344 rats. *Phytother Res* **16**, 712-718.

Im, S. A., Oh, S. T., Song, S., Kim, M. R., Kim, D. S., Woo, S. S., Jo, T. H., Park, Y. I., and Lee, C. K. (2005). Identification of optimal molecular size of modified Aloe polysaccharides with maximum immunomodulatory activity. *Int Immunopharmacol* **5**, 271-279.

Imanishi, K., Ishiguro, T., Saito, H., and Suzuki, I. (1981). Pharmacological studies on a plant lectin, Aloctin A. I. Growth inhibition of mouse methylcholanthrene-induced fibrosarcoma (Meth A) in ascites form by Aloctin A. *Experientia* **37**, 1186-1187.

Imanishi, K., and Suzuki, I. (1984). Augmentation of natural cell-mediated cytotoxic reactivity of mouse lymphoid cells by aloctin A. *Int J Immunopharmacol* **6**, 539-543.

Imanishi, K., and Suzuki, I. (1986). Induction of nonspecific cell-mediated cytotoxic reactivity from non- immune spleen cells treated with aloctin A. *Int J Immunopharmacol* **8**, 781-787.

Imanishi, K., Tsukuda, K., and Suzuki, I. (1986). Augmentation of lymphokine-activated killer cell activity in vitro by aloctin A. *Int J Immunopharmacol* **8**, 855-858.

Ishii, Y., Takino, Y., Toyo'oka, T., and Tanizawa, H. (1998). Studies of aloe. VI. Cathartic effect of isobarbaloin. *Biol Pharm Bull* **21**, 1226-1227.

Ishii, Y., Tanizawa, H., and Takino, Y. (1987). Determination of barbaloin in rat serum. *Chemical and Pharmaceutical Bulletin* **35**, 4642 - 4644.

Ishii, Y., Tanizawa, H., and Takino, Y. (1990). Studies of aloe. III. Mechanism of cathartic effect. (2). *Chem Pharm Bull (Tokyo)* **38**, 197-200.

Ishii, Y., Tanizawa, H., and Takino, Y. (1993). Rat selection test with respect to laxative activity induced by barbaloin. *Biol Pharm Bull* **16**, 1040.

Ishii, Y., Tanizawa, H., and Takino, Y. (1994). Studies of aloe. V. Mechanism of cathartic effect. (4). *Biol Pharm Bull* **17**, 651-653.

Izzo, A. A., Mascolo, N., and Capasso, F. (1998). Nitric oxide as a modulator of intestinal water and electrolyte transport. *Dig Dis Sci* **43**, 1605-1620.

Izzo, A. A., Sautebin, L., Borrelli, F., Longo, R., and Capasso, F. (1999). The role of nitric oxide in aloe-induced diarrhoea in the rat. *Eur J Pharmacol* **368**, 43-48.

Jain, A., Gupta, Y., and Jain, S. K. (2007). Perspectives of biodegradable natural polysaccharides for site-specific drug delivery to the colon. *J Pharm Pharm Sci* **10**, 86-128.

Johnson, R. B., Peterson, D. A., and Tolbert, B. M. (1960). Cellulose meatolism in the rat. *Journal of Nutrition* **72**, 353-356.

Joshi, S. P. (1998). Chemical constituents and biological activity of Aloe barbadensis-a review. *Journal of Medicinal and Aromatic Plant Sciences* **20**, 768-773.

Kahlon, J. B., Kemp, M. C., Yawei, N., Carpenter, R. H., Shannon, W. M., and McAnalley, B. H. (1991). In vitro evaluation of the synergistic antiviral effects of acemannan in combination with azidothymidine and acyclovir. *Mol Biother* **3**, 214-223.

Kai, M., Hayashi, K., Kaida, I., Aki, H., and Yamamoto, M. (2002). Permeation-enhancing effect of aloe-emodin anthrone on water-soluble and poorly permeable compounds in rat colonic mucosa. *Biol Pharm Bull* **25**, 1608-1613.

Karaca, K., Sharma, J. M., and Nordgren, R. (1995). Nitric oxide production by chicken macrophages activated by Acemannan, a complex carbohydrate extracted from Aloe vera. *Int J Immunopharmacol* **17**, 183-188.

Keum, Y. S., Park, K. K., Lee, J. M., Chun, K. S., Park, J. H., Lee, S. K., Kwon, H., and Surh, Y. J. (2000). Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. *Cancer Lett* **150**, 41-48.

Kilgore, S. P., Sigel, J. E., and Goldblum, J. R. (2000). Hyperplastic-like mucosal change in Crohn's disease: an unusual form of dysplasia? *Mod Pathol* **13**, 797-801.

Kim, H. S., Kacew, S., and Lee, B. M. (1999). In vitro chemopreventive effects of plant polysaccharides (Aloe barbadensis miller, Lentinus edodes, Ganoderma lucidum and Coriolus versicolor). *Carcinogenesis* **20**, 1637-1640.

Kim, H. S., and Lee, B. M. (1997). Inhibition of benzo[a]pyrene-DNA adduct formation by Aloe barbadensis Miller. *Carcinogenesis* **18**, 771-776.

Klein, A. D., and Penneys, N. S. (1988). Aloe vera [published erratum appears in J Am Acad Dermatol 1988 Jul;19(1 Pt 1):82]. *J Am Acad Dermatol* **18**, 714-720.

Klepser, T. B., Doucette, W. R., Horton, M. R., Buys, L. M., Ernst, M. E., Ford, J. K., Hoehns, J. D., Kautzman, H. A., Logemann, C. D., Swegle, J. M., Ritho, M., and Klepser, M. E. (2000). Assessment of patients' perceptions and beliefs regarding herbal therapies. *Pharmacotherapy* **20**, 83-87.

Koch, A. (1996). Metabolism of aloin--the influence of nutrition. *J Pharm Biomed Anal* **14**, 1335-1338.

Kodama, M., Kamioka, Y., Nakayama, T., Nagata, C., Morooka, N., and Ueno, Y. (1987). Generation of free radical and hydrogen peroxide from 2-hydroxyemodin, a direct-acting mutagen, and DNA strand breaks by active oxygen. *Toxicol Lett* **37**, 149-156.

Krumbiegel, G., and Schulz, H. U. (1993). Rhein and aloe-emodin kinetics from senna laxatives in man. *Pharmacology* **47 Suppl 1**, 120-124.

Kune, S., Kune, G. A., and Watson, L. (1986). The Melbourne colorectal cancer study: incidence findings by age, sex, site, migrants and religion. *Int J Epidemiol* **15**, 483-493.

Kuo, P. L., Lin, T. C., and Lin, C. C. (2002). The antiproliferative activity of aloe-emodin is through p53-dependent and p21-dependent apoptotic pathway in human hepatoma cell lines. *Life Sci* **71**, 1879-1892.

Lang, W. (1993). Pharmacokinetic-metabolic studies with 14C-aloe emodin after oral administration to male and female rats. *Pharmacology* **47 Suppl 1**, 110-119.

Langmead, L., Makins, R. J., and Rampton, D. S. (2004). Anti-inflammatory effects of aloe vera gel in human colorectal mucosa in vitro. *Aliment Pharmacol Ther* **19**, 521-527.

Lee, H. Z., Hsu, S. L., Liu, M. C., and Wu, C. H. (2001a). Effects and mechanisms of aloe-emodin on cell death in human lung squamous cell carcinoma. *Eur J Pharmacol* **431**, 287-295.

Lee, J. K., Lee, M. K., Yun, Y. P., Kim, Y., Kim, J. S., Kim, Y. S., Kim, K., Han, S. S., and Lee, C. K. (2001b). Acemannan purified from Aloe vera induces phenotypic and functional maturation of immature dendritic cells. *Int Immunopharmacol* **1**, 1275-1284.

Lee, K. Y., Weintraub, S. T., and Yu, B. P. (2000). Isolation and identification of a phenolic antioxidant from Aloe barbadensis. *Free Radical Biology and Medicine* **28**, 261-265.

Lee, M. J., Lee, O. H., Yoon, S. H., Lee, S. K., Chung, M. H., Park, Y. I., Sung, C. K., Choi, J. S., and Kim, K. W. (1998). In vitro angiogenic activity of Aloe vera gel on calf pulmonary artery endothelial (CPAE) cells. *Arch Pharm Res* **21**, 260-265.

Lee, Y. S. (1988). Background mucosal changes in colorectal carcinomas. *Cancer* **61**, 1563-1570.

Leegwater, D. C., de Groot, A. P., and van Kalmthout-Kuyper, M. (1974). The aetiology of caecal enlargement in the rat. *Food Cosmet Toxicol* **12**, 687-697.

Lim, B. O., Seong, N. S., Choue, R. W., Kim, J. D., Lee, H. Y., Kim, S. Y., Yu, B. P., Jeon, T. I., and Park, D. K. (2003). Efficacy of dietary aloe vera supplementation on hepatic cholesterol and oxidative status in aged rats. *J Nutr Sci Vitaminol (Tokyo)* **49**, 292-296.

Lissoni, P., Rovelli, F., Brivio, F., Zago, R., Colciago, M., Messina, G., Mora, A., and Porro, G. (2009). A randomized study of chemotherapy versus biochemotherapy with chemotherapy plus Aloe arborescens in patients with metastatic cancer. *In Vivo* **23**, 171-175.

Lupton, J. R. (2004). Microbial degradation products influence colon cancer risk: the butyrate controversy. *J Nutr* **134**, 479-482.

Luyckx, V. A., Ballantine, R., Claeys, M., Cuyckens, F., Van den Heuvel, H., Cimanga, R. K., Vlietinck, A. J., De Broe, M. E., and Katz, I. J. (2002). Herbal remedy-associated acute renal failure secondary to Cape aloes. *Am J Kidney Dis* **39**, E13.

Lyden-Sokolowski, A., Nilsson, A., and Sjoberg, P. (1993). Two-year carcinogenicity study with sennosides in the rat: emphasis on gastro-intestinal alterations. *Pharmacology* **47 Suppl 1**, 209-215.

MacFie, J. (2004). Current status of bacterial translocation as a cause of surgical sepsis. *Br Med Bull* **71**, 1-11.

Mandal, G., and Das, A. (1980). Structure of the D-galactan isolated from *Aloe barbadensis* Miller. *Carbohydrate Research* **86**, 247-257.

Manitto, P., Monti, D., and Speranza, G. (1990). Studies on Aloe. Part 6. Conformation and absolute configuration of Aloins A and B and related 10-*C*-Glucosyl-9-anthrones. *Journal of the Chemical Society Perkin Trans I* **5**, 1297-1300.

Mapp, R. K., and McCarthy, T. J. (1970). The assessment of purgative principles in aloes. *Planta Med* **18**, 361-365.

Marshall, J. M. (1990). Aloe vera gel: what is the evidence? *Pharm J* **244**, 360-362.

Mascolo, N., Izzo, A. A., Boelli, F., Capasso, R., DiCarlo, G., Sautebin, L., and Capasso, F. (2004). Healing powers of aloes. In *Aloes: the genus Aloe.* (T. Reynolds, Ed.), pp. 209-238. CRC Press, Boga Raton, FL.

Matsuda, Y., Yokohira, M., Suzuki, S., Hosokawa, K., Yamakawa, K., Zeng, Y., Ninomiya, F., Saoo, K., Kuno, T., and Imaida, K. (2008). One-year chronic toxicity study of Aloe arborescens Miller var. natalensis Berger in Wistar Hannover rats. A pilot study. *Food Chem Toxicol* **46**, 733-739.

Mijatovic, S., Maksimovic-Ivanic, D., Radovic, J., Miljkovic, D., Harhaji, L., Vuckovic, O., Stosic-Grujicic, S., Mostarica Stojkovic, M., and Trajkovic, V. (2005). Anti-glioma action of aloe emodin: the role of ERK inhibition. *Cell Mol Life Sci* **62**, 589-598.

Mijatovic, S., Maksimovic-Ivanic, D., Radovic, J., Popadic, D., Momcilovic, M., Harhaji, L., Miljkovic, D., and Trajkovic, V. (2004). Aloe-emodin prevents cytokine-induced tumor cell death: the inhibition of auto-toxic nitric oxide release as a potential mechanism. *Cell Mol Life Sci* **61**, 1805-1815.

Moon, E. J., Lee, Y. M., Lee, O. H., Lee, M. J., Lee, S. K., Chung, M. H., Park, Y. I., Sung, C. K., Choi, J. S., and Kim, K. W. (1999). A novel angiogenic factor derived from Aloe vera gel: beta-sitosterol, a plant sterol. *Angiogenesis* **3**, 117-123.

Morgenstern, K. (2009). Plant Profile: Aloe vera. http://www.sacredearth.com/ethnobotany/plantprofiles/aloe.php.

Mori, H., Sugie, S., Niwa, K., Takahashi, M., and Kawai, K. (1985). Induction of intestinal tumours in rats by chrysazin. *Br J Cancer* **52**, 781-783.

Mori, H., Yoshimi, N., Iwata, H., Mori, Y., Hara, A., Tanaka, T., and Kawai, K. (1990). Carcinogenicity of naturally occurring 1-hydroxyanthraquinone in rats: induction of large bowel, liver and stomach neoplasms. *Carcinogenesis* **11**, 799-802.

Mueller, S. O., Eckert, I., Lutz, W. K., and Stopper, H. (1996). Genotoxicity of the laxative drug components emodin, aloe-emodin and danthron in mammalian cells: topoisomerase II mediated? *Mutat Res* **371**, 165-173.

Mueller, S. O., Lutz, W. K., and Stopper, H. (1998a). Factors affecting the genotoxic potency ranking of natural anthraquinones in mammalian cell culture systems. *Mutat Res* **414**, 125-129.

Mueller, S. O., and Stopper, H. (1999). Characterization of the genotoxicity of anthraquinones in mammalian cells. *Biochim Biophys Acta* **1428**, 406-414.

Mueller, S. O., Stopper, H., and Dekant, W. (1998b). Biotransformation of the anthraquinones emodin and chrysophanol by cytochrome P450 enzymes. Bioactivation to genotoxic metabolites. *Drug Metab Dispos* **26**, 540-546.

Nascimbeni, R., Donato, F., Ghirardi, M., Mariani, P., Villanacci, V., and Salerni, B. (2002). Constipation, anthranoid laxatives, melanosis coli, and colon cancer: a risk assessment using aberrant crypt foci. *Cancer Epidemiol Biomarkers Prev* **11**, 753-757.

Nath, D., Sethi, N., Singh, R. K., and Jain, A. K. (1992). Commonly used Indian abortifacient plants with special reference to their terratologic effects in rats. *Journal of Ethnopharmacology* **36**, 147-154.

Neuhouser, M. L. (2003). Dietary supplement use by American women: challenges in assessing patterns of use, motives and costs. *J Nutr* **133**, 1992S-1996S.

Ni, Y., Turner, D., Yates, K. M., and Tizard, I. (2004). Isolation and characterization of structural components of Aloe vera L. leaf pulp. *Int Immunopharmacol* **4**, 1745-1755.

Nishikawa, A., Kase, Y., Hayakawa, T., Yanagisawa, T., Kanno, J., and Hayashi, Y. (1997). Enhancement of cell proliferation and prostaglandin biosynthesis by 1,8-dihydroxyanthraquinone in the rat large intestine. *Carcinogenesis* **18**, 1259-1263.

NTP (2006). Specifications for the Conduct of Studies to Evaluate the Toxic and Carcinogenic Potential of Chemical, Biological and Physical Agents in Laboratory Animals for the National Toxicology Program (NTP). National Institutes of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services, National Toxicology Program Research Triangle Park, NC.

NTP (2008). Photococarcinogenesis Study of Aloe Vera [CAS NO. 481-72-1 (Aloe-emodin)] in SKH-1 Mice (Simulated Solar Light and Topical Application Study). In *Technical Report Series No. 553. NIH Publication No. 08-5894.* National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.

Okamura, N., Asaia, M., Hinea, N., and Yagi, A. (1996). High-performance liquid chromatographic determination of phenolic compounds in Aloe species. *J Chromatogr A* **746**, 225-231.

Okamura, N., Hine, N., Harada, S., Fujioka, T., Mihashi, K., Nishi, M., Kazumoto, M., and Yagi, A. (1997). Diastereomic C-glycosylanthrones of *Aloe vera* leaves. *Phytochemistry* **45**, 1519-1522.

Olubuyide, I. O., Williamson, R. C., Bristol, J. B., and Read, A. E. (1984). Goblet cell hyperplasia is a feature of the adaptive response to jejunoileal bypass in rats. *Gut* **25**, 62-68.

Paez, A., Gebre, G. M., Gonzalez, M. E., and Tschaplinski, T. J. (2000). Growth, soluble carbohydrates, and aloin concentration of Aloe vera plants exposed to three irradiance levels. *Environmental and Experimental Botany* **44**, 133-139.

Park, M. K., Park, J. H., Kim, N. Y., Shin, Y. G., Choi, Y. S., Lee, J. G., Kim, K. H., and Lee, S. K. (1998). Analysis of 13 compounds in Aloe species by high performance liquid chromatography. *Phytochemical analysis* **9**, 186-191.

Parra, A. L., Yhebra, R. S., Sardinas, I. G., and Buela, L. I. (2001). Comparative study of the assay of Artemia salina L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. *Phytomedicine* **8**, 395-400.

Pecere, T., Gazzola, M. V., Mucignat, C., Parolin, C., Vecchia, F. D., Cavaggioni, A., Basso, G., Diaspro, A., Salvato, B., Carli, M., and Palu, G. (2000). Aloe-emodin is a new type of anticancer agent with selective activity against neuroectodermal tumors. *Cancer Res* **60**, 2800-2804.

Pelley, R. P., Martini, W. J., Liu, D. Q., Yang, Z., Rachui, S., Keuei-Mei Li, T. A. W., and Strickland, F. M. (1998). Multiparameter analysis of commercial "aloe vera" materials and comparison to Aloe barbadensis Miller extracts. *Subtropical Plant Science* **50**, 1-14.

Perkins, J. G., Petersen, A. B., and Riley, J. A. (1950). Renal and cardiac lesions in potassium deficiency due to chronic diarrhea. *Am J Med* **8**, 115-123, illust.

Pogribna, M., Freeman, J. P., Paine, D., and Boudreau, M. D. (2008). Effect of Aloe vera whole leaf extract on short chain fatty acids production by Bacteroides fragilis, Bifidobacterium infantis and Eubacterium limosum. *Lett Appl Microbiol* **46**, 575-580.

Prior, P., Gyde, S. N., Macartney, J. C., Thompson, H., Waterhouse, J. A., and Allan, R. N. (1982). Cancer morbidity in ulcerative colitis. *Gut* **23**, 490-497.

Pugh, N., Ross, S. A., ElSohly, M. A., and Pasco, D. S. (2001). Characterization of Aloeride, a new high-molecular-weight polysaccharide from Aloe vera with potent immunostimulatory activity. *J Agric Food Chem* **49**, 1030-1034.

Qiu, Z., Jones, K., Wylie, M., Jia, Q., and Orndorff, S. (2000). Modified Aloe barbadensis polysaccharide with immunoregulatory activity. *Planta Med* **66**, 152-156.

Rabe, C., Musch, A., Schirmacher, P., Kruis, W., and Hoffmann, R. (2005). Acute hepatitis induced by an Aloe vera preparation: a case report. *World J Gastroenterol* **11**, 303-304.

Rajasekaran, S., Sivagnanam, K., and Subramanian, S. (2005). Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. *Pharmacol Rep* **57**, 90-96.

Ramamoorthy, L., Kemp, M. C., and Tizard, I. R. (1996). Acemannan, a beta-(1,4)-acetylated mannan, induces nitric oxide production in macrophage cell line RAW 264.7. *Mol Pharmacol* **50**, 878-884.

Ramamoorthy, L., and Tizard, I. R. (1998). Induction of apoptosis in a macrophage cell line RAW 264.7 by acemannan, a beta-(1,4)-acetylated mannan. *Mol Pharmacol* **53**, 415-421.

Reynolds, T. (1985). The compounds in aloe leaf exudates: a review. *Botanical Journal of the Linnean society* **90**, 157-178.

Robson, M. C., Heggers, J. P., and Hagstrom, W. J. (1982). Myth, magic, witchcraft or fact? Aloe vera revisited. *J Burn Care Rehabil* **3**, 157-162.

Rodrigues, O. R., Antonangelo, L., Yagi, N., Minamoto, H., Schmidt Junior, A. F., Capelozzi, V. L., Goldenberg, S., and Saldiva, P. H. (1997). Prognostic significance of argyrophilic nucleolar organizer region (AgNOR) in resected non-small cell lung cancer (NSCLC). *Jpn J Clin Oncol* **27**, 298-304.

Roman-Ramos, R., Flores-Saenz, J. L., Partida-Hernandez, G., Lara-Lemus, A., and Alarcon-Aguilar, F. (1991). Experimental study of the hypoglycemic effect of some antidiabetic plants. *Arch Invest Med (Mex)* **22**, 87-93.

Ross, S. A., ElSohly, M. A., and Wilkins, S. P. (1997). Quantitative analysis of Aloe vera mucilaginous polysaccharide in commercial Aloe vera products. *J AOAC Ineternational* **80**, 455-457.

Saada, H. N., Ussama, Z. S., and Mahdy, A. M. (2003). Effectiveness of Aloe vera on the antioxidant status of different tissues in irradiated rats. *Pharmazie* **58**, 929-931.

Saccu, D., Bogoni, P., and Procida, G. (2001). Aloe exudate: characterization by reversed phase HPLC and headspace GC-MS. *J Agric Food Chem* **49**, 4526-4530.

Saito, H., Imanishi, K., and Okabe, S. (1989). [Effects of aloe extracts, aloctin A, on gastric secretion and on experimental gastric lesions in rats]. *Yakugaku Zasshi* **109**, 335-339.

Saleem, R., Faizi, S., Deeba, F., Siddiqui, B. S., and Qazi, M. H. (1997). Anthrones from *Aloe barbadensis*. *Phytochemistry* **45**, 1279-1282.

Schorkhuber, M., Richter, M., Dutter, A., Sontag, G., and Marian, B. (1998). Effect of anthraquinone-laxatives on the proliferation and urokinase secretion of normal, premalignant and malignant colonic epithelial cells. *Eur J Cancer* **34**, 1091-1098.

Sendelbach, L. E. (1989). A review of the toxicity and carcinogenicity of anthraquinone derivatives. *Toxicology* **57**, 227-240.

Shah, A. H., Quereshi, S., Tariq, M., and Ageel, A. M. (1989). Toxicity studies on six plants used in the traditional arab system of medicine. *Phytother Res* **3**, 25-28.

Shelton, R. M. (1991). Aloe vera. Its chemical and therapeutic properties [see comments]. *Int J Dermatol* **30**, 679-683.

Shimpo, K., Beppu, H., Chihara, T., Kaneko, T., Shinzato, M., and Sonoda, S. (2006). Effects of aloe arborescens ingestion on azoxymethane-induced intestinal carcinogenesis and hematological and biochemical parameters of male F344 rats. *Asian Pac J Cancer Prev* **7**, 585-590.

Shimpo, K., Chihara, T., Beppu, H., Ida, C., Kaneko, T., Nagatsu, T., and Kuzuya, H. (2001). Inhibition of azoxymethane-induced aberrant crypt foci formation in rat colorectum by whole leaf Aloe arborescens Miller var. natalensis Berger. *Phytother Res* **15**, 705-711.

Siegers, C. P., von Hertzberg-Lottin, E., Otte, M., and Schneider, B. (1993). Anthranoid laxative abuse--a risk for colorectal cancer? *Gut* **34**, 1099-1101.

Sigel, J. E., Petras, R. E., Lashner, B. A., Fazio, V. W., and Goldblum, J. R. (1999). Intestinal adenocarcinoma in Crohn's disease: a report of 30 cases with a focus on coexisting dysplasia. *Am J Surg Pathol* **23**, 651-655.

Simal, S., Femenía, A., Llull, P., and Rosselló, C. (2000). Dehydration of aloe vera: simulation of drying curves and evaluation of functional properties. *J Food Engineering* **43**, 109-114.

Smits-Van Prooije, A. E., De Groot, A. P., Dreef-Van der Meulen, H. C., and Sinkeldam, E. J. (1990). Chronic toxicity and carcinogenicity study of isomalt in rats and mice. *Food Chem Toxicol* **28**, 243-251.

Spoerke, D. G., and Ekins, B. R. (1980). Aloe vera--fact or quackery. *Vet Hum Toxicol* **22**, 418-424.

Stevens, C. E., and Hume, I. D. (1998). Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. *Physiol Rev* **78**, 393-427.

Stolk, L. M., and Hoogtanders, K. (1999). Detection of laxative abuse by urine analysis with HPLC and diode array detection. *Pharm World Sci* **21**, 40-43.

Takahashi, M., Konaka, D., Sakamoto, A., and Morikawa, H. (2005). Nocturnal uptake and assimilation of nitrogen dioxide by C3 and CAM plants. *Z Naturforsch [C]* **60**, 279-284.

Talmadge, J., Chavez, J., Jacobs, L., Munger, C., Chinnah, T., Chow, J. T., Williamson, D., and Yates, K. (2004). Fractionation of Aloe vera L. inner gel, purification and molecular profiling of activity. *Int Immunopharmacol* **4**, 1757-1773.

Tanaka, M., Misawa, E., Ito, Y., Habara, N., Nomaguchi, K., Yamada, M., Toida, T., Hayasawa, H., Takase, M., Inagaki, M., and Higuchi, R. (2006). Identification of five phytosterols from Aloe vera gel as anti-diabetic compounds. *Biol Pharm Bull* **29**, 1418-1422.

Tanaka, T. (2009). Colorectal carcinogenesis: Review of human and experimental animal studies. *J Carcinog* **8**, 5.

Teradaira, R., Shinzato, M., Beppu, H., and Fujita, H. (1993). Antigastric ulcer effects in rats of aloe arborescens miller var. natalensis berger extract. *Phytother Res* **7**, S34-S36.

Til, H. P., Feron, V. J., Immel, H. R., and Vogel, W. F. (1986). Chronic (89-week) feeding study with hydroxypropyl distarch phosphate, starch acetate, lactose and sodium alginate in mice. *Food Chem Toxicol* **24**, 825-834.

Turner, C. E., Williamson, D. A., Stroud, P. A., and Talley, D. J. (2004). Evaluation and comparison of commercially available Aloe vera L. products using size exclusion chromatography with refractive index and multi-angle laser light scattering detection. *Int Immunopharmacol* **4**, 1727-1737.

Vaiphei, K., Saha, M., Sharma, B. C., Bhasin, D. K., and Singh, K. (2004). Goblet cell status in idiopathic ulcerative colitis--implication in surveillance program. *Indian J Pathol Microbiol* **47**, 16-21.

van Gorkom, B. A., de Vries, E. G., Karrenbeld, A., and Kleibeuker, J. H. (1999). Review article: anthranoid laxatives and their potential carcinogenic effects. *Aliment Pharmacol Ther* **13**, 443-452.

van Zyl, A., Meyer, A. J., and van der Merwe, M. (1999). The influence of fibre in the diet on growth rates and the digestibility of nutrients in the greater cane rat (Thryonomys swinderianus). *Comp Biochem Physiol A Mol Integr Physiol* **123**, 129-135.

Vargas, F., Fraile, G., Velasquez, M., Correia, H., Fonseca, G., Marin, M., Marcano, E., and Sanchez, Y. (2002). Studies on the photostability and phootoxicity of aloe-emodin, emodin and rhein. *Pharmazie* **57**, 399-404.

Vath, P., Wamer, W. G., and Falvey, D. E. (2002). Photochemistry and phototoxicity of aloe emodin. *Photochem Photobiol* **75**, 346-352.

Vázquez, B., Avila, G., Segura, D., and Escalante, B. (1996). Antiinflammatory activity of extracts from Aloe vera gel. *J Ethnopharmacol* **55**, 69-75.

Viljoen, A. M., and Van Wyk, B. (2000). The chemotaxonomic significance of the phenyl pyrone aloenin in the genus Aloe. *Biochem Syst Ecol* **28**, 1009-1017.

Vogelzang, J. L. (2001). What you need to know about dietary supplements. *Home Healthcare Nurse* **19**, 50-52.

Vogler, B. K., and Ernst, E. (1999). Aloe vera: a systematic review of its clinical effectiveness. *Br J Gen Pract* **49**, 823-828.

Vyth, A., and Kamp, P. E. (1979). Detection of anthraquinone laxatives in the urine. *Pharm Weekbl* **114**, 456-459.

Wadman, M. (2009). Centre turns away from healing herbs. *Nature* **462**, 711.

Waller, T. A., Pelley, R. P., and Strickland, F. M. (2004). Industrial processing and quality control of Aloe barbadensis. In *Aloes: The Genus Aloes* (T. Reynolds, Ed.), pp. 139-205. CRC Press, Baca Raton, FL.

Wamer, W. G., Vath, P., and Falvey, D. E. (2003). In vitro studies on the photobiological properties of aloe emodin and aloin A. *Free Radical Biology and Medicine* **34**, 233-242.

Wang, Y.-T., and Strong, K. J. (1995). Two-year study monitoring several physical and chemical properties of field-grown aloe barbadensis Miller leaves. *Subtropical Plant Science* **47**, 34-38.

Werner, C. (2007). Committee on Herbal Medicinal Products (HMPC). Assessment report on *Aloe Barbadensis* Miller and *Aloe* (various species, mainly *Aloe Ferox* Miller and its hybrids. (B. Mertz, Ed.). European Medicines Agency, Evaluation of Medicines for human use, London, England.

Westendorf, J., Marquardt, H., Poginsky, B., Dominiak, M., and Schmidt, J. (1990). Genotoxicity of naturally occurring hydroxyanthraquinones. *Mutat Res* **240**, 1-12.

Willems, M., van Buuren, H. R., and de Krijger, R. (2003). Anthranoid self-medication causing rapid development of melanosis coli. *Neth J Med* **61**, 22-24.

Williams, Y. (2007). How to prepare Aloe herbal treatments. http://www.unexplainable.net/artman/publish/article_6930.shtml. Unexplainable.Net.

Williamson, R. C., Davies, P. W., Bristol, J. B., and Wells, M. (1982). Intestinal adaptation and experimental carcinogenesis after partial colectomy. Increased tumour yields are confined to the anastomosis. *Gut* **23**, 316-325.

Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* **64**, 3-19.

Winters, W. D., Benavides, R., and Clouse, W. J. (1981). Effects of aloe extracts on human normal and tumor cells in vitro. *Economic Botany* **35**, 89-95.

Wolfe, D., Schmutte, C., Westendorf, J., and Marquardt, H. (1990). Hydroxyanthraquinones as tumor promoters: enhancement of malignant transformation of C3H mouse fibroblasts and growth stimulation of primary rat hepatocytes. *Cancer Res* **50**, 6540-6544.

Yagi, A., Egusa, T., Arase, M., Tanabe, M., and Tsuji, H. (1997). Isolation and characterization of the glycoprotein fraction with a proliferation-promoting activity on human and hamster cells in vitro from Aloe vera gel. *Planta Med* **63**, 18-21.

Yagi, A., Nakamori, J., Yamada, T., Iwase, H., Tanaka, T., Kaneo, Y., Qiu, J., and Orndorff, S. (1999). In vivo metabolism of aloemannan. *Planta Med* **65**, 417-420.

Yagi, T., and Yamauchi, K. (1999). Synergistic effects of anthraquinones on the purgative activity of rhein anthrone in mice. *J Pharm Pharmacol* **51**, 93-95.

Yang, M. G., Manoharan, K., and Young, A. K. (1969). Influence and degradation of dietary cellulose in cecum of rats. *J Nutr* **97**, 260-264.

Yaron, A. (1993). Characterization of aloe vera gel before and after autodegradation, and stabilization of the natural fresh gel. *Phytother Res* **7**, 11-13.

Yeh, F. T., Wu, C. H., and Lee, H. Z. (2003). Signaling pathway for aloe-emodin-induced apoptosis in human H460 lung nonsmall carcinoma cell. *Int J Cancer* **106**, 26-33.

Yen, G.-C., Duh, P.-D., and Chuand, D.-Y. (2000). Antioxidant activity of anthraquinones and anthrone. *Food Chemistry* **70**, 437-441.

Yokohira, M., Matsuda, Y., Suzuki, S., Hosokawa, K., Yamakawa, K., Hashimoto, N., Saoo, K., Nabae, K., Doi, Y., Kuno, T., and Imaida, K. (2009). Equivocal colonic carcinogenicity of Aloe arborescens Miller var. natalensis berger at high-dose level in a Wistar Hannover rat 2-y study. *J Food Sci* **74**, T24-30.

Yongchaiyudha, S., Rungpitarangsi, V., Bunyaprophatsara, N., and Chokechaijaroenporn, O. (1996). Antidiabetic activity of Aloe vera L juice. I. Clinical trial in new cases of diabetes mellitus. *Phytomedicine* **3**, 241-243.

Zhang, L., and Tizard, I. R. (1996). Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from Aloe vera gel. *Immunopharmacology* **35**, 119-128.

Zhao, J., Wang, J., Chen, Y., and Agarwal, R. (1999). Anti-tumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidin B5-3'-gallate as the most effective antioxidant constituent. *Carcinogenesis* **20**, 1737-1745.

Zonta, F., Bogoni, P., Masotti, P., and Micali, G. (1995). High-performance liquid chromatographic profiles of aloe constituents and determination of aloin in beverages, with reference to the EEC regulation for flavouring substances. *J Chromatogr A* **718**, 99-106.

APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF ALOE VERA WHOLE LEAF EXTRACT

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract[a]

	0%	0.5%	1.0%	1.5%
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund sacrifice	28	26	23	26
Natural deaths	2	5	2	4
Survivors				
Moribund sacrifice	3		4	3
Terminal sacrifice	15	17	19	15
Animals examined microscopically	48	48	48	48
Alimentary System				
Esophagus	(48)	(48)	(48)	(48)
Intestine large, ascending colon	(47)	(47)	(48)	(46)
Adenoma			19 (40%)	8 (17%)
Carcinoma			4 (8%)	8 (17%)
Leiomyoma			1 (2%)	
Leukemia mononuclear		1 (2%)		1 (2%)
Intestine large, cecum	(46)	(45)	(48)	(48)
Adenoma			8 (17%)	7 (15%)
Adenoma, multiple				1 (2%)
Carcinoma			1 (2%)	2 (4%)
Leiomyosarcoma				1 (2%)
Leukemia mononuclear	2 (4%)			3 (6%)
Lymphoid tissue, leukemia mononuclear		1 (2%)		
Intestine large, colon	(0)	(1)	(3)	(5)
Adenoma			1 (33%)	2 (40%)
Carcinoma			1 (33%)	
Intestine large, descending colon	(47)	(46)	(46)	(47)
Carcinoma			1 (2%)	
Leukemia mononuclear				1 (2%)
Intestine large, rectum	(47)	(47)	(48)	(48)
Intestine large, transverse colon	(47)	(47)	(47)	(47)
Adenoma			6 (13%)	3 (6%)
Carcinoma			1 (2%)	1 (2%)
Leukemia mononuclear		1 (2%)		1 (2%)
Intestine small	(0)	(1)	(0)	(0)
Mesothelioma malignant		1 (100%)		
Intestine small, duodenum	(48)	(46)	(48)	(48)
Leukemia mononuclear				1 (2%)
Intestine small, ileum	(45)	(45)	(48)	(48)
Leukemia mononuclear	1 (2%)			2 (4%)
Intestine small, jejunum	(45)	(44)	(46)	(46)
Carcinoma		1 (2%)		
Leukemia mononuclear				2 (4%)
Liver	(48)	(48)	(48)	(48)
Cholangiocarcinoma		1 (2%)		
Hepatocellular adenoma	5 (10%)	1 (2%)		
Hepatocellular carcinoma	3 (6%)			1 (2%)
Histiocytic sarcoma	1 (2%)	1 (2%)		
Leukemia mononuclear	26 (54%)	19 (40%)	20 (42%)	22 (46%)
Mesentery	(10)	(10)	(4)	(5)
Leukemia mononuclear	1 (10%)	1 (10%)	1 (25%)	
Mesothelioma malignant		2 (20%)		1 (20%)
Oral mucosa	(2)	(1)	(2)	(1)
Sarcoma			1 (50%)	
Squamous cell papilloma		1 (100%)	1 (50%)	1 (100%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Alimentary System (continued)				
Pancreas	(48)	(48)	(48)	(48)
Leukemia mononuclear	2 (4%)	3 (6%)	2 (4%)	4 (8%)
Mesothelioma malignant		1 (2%)		
Mixed tumor benign		1 (2%)		
Acinar cell, adenoma		1 (2%)		1 (2%)
Proximal Colon	(44)	(44)	(46)	(41)
Adenoma			7 (15%)	10 (24%)
Carcinoma			4 (9%)	4 (10%)
Leukemia mononuclear				1 (2%)
Salivary glands	(48)	(48)	(48)	(48)
Leukemia mononuclear		1 (2%)	2 (4%)	
Sublingual gland, adenoma		1 (2%)		
Stomach, forestomach	(48)	(47)	(48)	(48)
Squamous cell papilloma		1 (2%)		
Stomach, glandular	(48)	(47)	(48)	(48)
Leukemia mononuclear				2 (4%)
Cardiovascular System				
Blood vessel	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)			
Heart	(48)	(48)	(48)	(48)
Leukemia mononuclear	14 (29%)	10 (21%)	8 (17%)	8 (17%)
Schwannoma malignant	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Pericardium, osteosarcomas, metastatic, bone		1 (2%)		
Endocrine System				
Adrenal cortex	(48)	(48)	(48)	(48)
Adenoma		1 (2%)	1 (2%)	
Leukemia mononuclear	3 (6%)	3 (6%)	4 (8%)	4 (8%)
Mesothelioma malignant		1 (2%)		
Adrenal medulla	(48)	(47)	(48)	(48)
Leukemia mononuclear	6 (13%)	5 (11%)	4 (8%)	4 (8%)
Pheochromocytoma benign	1 (2%)		1 (2%)	4 (8%)
Pheochromocytoma malignant	3 (6%)	10 (21%)	14 (29%)	7 (15%)
Bilateral, pheochromocytoma benign	2 (4%)		1 (2%)	
Bilateral, pheochromocytoma malignant	4 (8%)	2 (4%)	1 (2%)	1 (2%)
Islets, pancreatic	(48)	(48)	(48)	(48)
Adenoma	3 (6%)	4 (8%)	5 (10%)	4 (8%)
Leukemia mononuclear				3 (6%)
Parathyroid gland	(47)	(48)	(46)	(48)
Adenoma	1 (2%)	1 (2%)		1 (2%)
Pituitary gland	(48)	(48)	(47)	(48)
Leukemia mononuclear	5 (10%)	1 (2%)	3 (6%)	1 (2%)
Pars distalis, adenoma	30 (63%)	30 (63%)	27 (57%)	22 (46%)
Thyroid gland	(48)	(47)	(48)	(48)
Bilateral, c-cell, adenoma				1 (2%)
C-cell, adenoma	2 (4%)	7 (15%)	3 (6%)	2 (4%)
C-cell, carcinoma	1 (2%)	1 (2%)	3 (6%)	
Follicular cell, adenoma	2 (4%)	1 (2%)	1 (2%)	
Follicular cell, carcinoma			1 (2%)	
General Body System				
Tissue NOS	(0)	(0)	(1)	(2)
Mediastinum, leukemia mononuclear				1 (50%)
Scrotal, mesothelioma malignant			1 (100%)	1 (50%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Genital System				
Epididymis	(48)	(48)	(48)	(48)
Leukemia mononuclear				1 (2%)
Mesothelioma malignant		3 (6%)	1 (2%)	2 (4%)
Preputial gland	(48)	(48)	(48)	(48)
Adenoma	5 (10%)	4 (8%)	2 (4%)	1 (2%)
Carcinoma	2 (4%)		3 (6%)	1 (2%)
Squamous cell carcinoma	4 (8%)	2 (4%)		1 (2%)
Squamous cell papilloma		1 (2%)	3 (6%)	
Prostate	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)	1 (2%)		
Seminal vesicle	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)			1 (2%)
Mesothelioma malignant		2 (4%)		
Testes	(48)	(48)	(48)	(48)
Mesothelioma malignant		2 (4%)	1 (2%)	1 (2%)
Bilateral, interstitial cell, adenoma	27 (56%)	25 (52%)	28 (58%)	23 (48%)
Interstitial cell, adenoma	10 (21%)	14 (29%)	7 (15%)	11 (23%)
Hematopoietic System				
Bone marrow	(48)	(48)	(48)	(48)
Histiocytic sarcoma		1 (2%)		
Leukemia mononuclear	1 (2%)	3 (6%)	3 (6%)	6 (13%)
Lymph node	(24)	(13)	(19)	(17)
Leukemia mononuclear			2 (11%)	
Axillary, leukemia mononuclear	2 (8%)	1 (8%)	2 (11%)	1 (6%)
Brachial, leukemia mononuclear			1 (5%)	
Cervical, carcinoma, metastatic, thyroid gland			1 (5%)	
Deep cervical, leukemia mononuclear	1 (4%)	1 (8%)	2 (11%)	1 (6%)
Hepatic, leukemia mononuclear	1 (4%)			
Iliac, leukemia mononuclear			1 (5%)	
Inguinal, leukemia mononuclear	1 (4%)		1 (5%)	
Lumbar, histiocytic sarcoma	1 (4%)			
Lumbar, leukemia mononuclear	6 (25%)	1 (8%)	5 (26%)	1 (6%)
Mediastinal, histiocytic sarcoma	1 (4%)	1 (8%)		
Mediastinal, leukemia mononuclear	9 (38%)	3 (23%)	2 (11%)	5 (29%)
Pancreatic, leukemia mononuclear	7 (29%)	2 (15%)	5 (26%)	7 (41%)
Renal, histiocytic sarcoma		1 (8%)		
Renal, leukemia mononuclear	4 (17%)	2 (15%)	2 (11%)	3 (18%)
Lymph node, mandibular	(48)	(48)	(48)	(48)
Histiocytic sarcoma	1 (2%)			
Leukemia mononuclear	11 (23%)	7 (15%)	8 (17%)	6 (13%)
Lymph node, mesenteric	(47)	(48)	(48)	(48)
Leukemia mononuclear	10 (21%)	10 (21%)	9 (19%)	7 (15%)
Spleen	(48)	(48)	(48)	(48)
Hemangiosarcoma		1 (2%)		
Histiocytic sarcoma	1 (2%)			
Leukemia mononuclear	26 (54%)	20 (42%)	23 (48%)	24 (50%)
Mesothelioma malignant		2 (4%)		
Thymus	(46)	(47)	(44)	(44)
Leukemia mononuclear	8 (17%)	3 (6%)	6 (14%)	5 (11%)
Integumentary System				
Mammary gland	(44)	(46)	(48)	(41)
Adenocarcinoma		1 (2%)	1 (2%)	
Fibroadenoma	1 (2%)	4 (9%)	3 (6%)	1 (2%)
Leukemia mononuclear	1 (2%)	1 (2%)		1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Integumentary System (continued)				
Skin	(48)	(48)	(48)	(48)
Basal cell carcinoma		1 (2%)		
Fibroma	4 (8%)	1 (2%)	3 (6%)	1 (2%)
Granular cell tumor benign	1 (2%)			
Hemangiosarcoma			1 (2%)	
Keratoacanthoma	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Leukemia mononuclear			1 (2%)	2 (4%)
Lipoma	1 (2%)		1 (2%)	
Sarcoma				2 (4%)
Sebaceous gland, adenoma		1 (2%)		
Subcutaneous tissue, fibroma		1 (2%)		
Subcutaneous tissue, lipoma			1 (2%)	
Subcutaneous tissue, sarcoma			1 (2%)	
Musculoskeletal System				
Bone	(0)	(2)	(2)	(1)
Chondrosarcoma			1 (50%)	
Osteoma		1 (50%)		
Rib, osteosarcoma		1 (50%)		
Bone, femur	(48)	(48)	(48)	(48)
Skeletal muscle	(2)	(2)	(0)	(2)
Mesothelioma malignant		1 (50%)		
Nervous System				
Brain	(0)	(0)	(1)	(0)
Meninges, meningioma malignant			1 (100%)	
Brain, brain stem	(48)	(48)	(48)	(48)
Leukemia mononuclear	5 (10%)	4 (8%)		6 (13%)
Brain, cerebellum	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)	3 (6%)		7 (15%)
Brain, cerebrum	(48)	(48)	(48)	(48)
Granular cell tumor benign	1 (2%)			
Leukemia mononuclear	2 (4%)	3 (6%)		5 (10%)
Spinal cord	(1)	(1)	(0)	(3)
Astrocytoma malignant				1 (33%)
Leukemia mononuclear		1 (100%)		
Respiratory System				
Lung	(48)	(48)	(48)	(48)
Alveolar/bronchiolar adenoma		5 (10%)		1 (2%)
Carcinoma, metastatic, thyroid gland			1 (2%)	
Chondrosarcoma, metastatic, bone			1 (2%)	
Histiocytic sarcoma	1 (2%)			
Leukemia mononuclear	24 (50%)	13 (27%)	13 (27%)	16 (33%)
Osteosarcoma, metastatic, bone		1 (2%)		
Sarcoma, metastatic, skin				1 (2%)
Nose	(48)	(48)	(47)	(48)
Leukemia mononuclear		1 (2%)		
Sarcoma, metastatic, oral mucosa			1 (2%)	
Trachea	(48)	(48)	(48)	(48)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Special Senses System				
Eye	(47)	(46)	(47)	(48)
Harderian Gland	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)			
Lacrimal gland	(0)	(1)	(0)	(0)
Squamous cell carcinoma, metastatic, Zymbal's gland		1 (100%)		
Zymbal's gland	(0)	(2)	(1)	(0)
Carcinoma			1 (100%)	
Squamous cell carcinoma		2 (100%)		
Urinary System				
Kidney	(48)	(48)	(48)	(48)
Leukemia mononuclear	4 (8%)	2 (4%)	2 (4%)	3 (6%)
Renal tubule, adenoma	1 (2%)			1 (2%)
Urinary bladder	(48)	(48)	(48)	(48)
Leukemia mononuclear	4 (8%)	2 (4%)	1 (2%)	
Transitional epithelium, papilloma	1 (2%)	1 (2%)		
Systemic Lesions				
Multiple Organs	(48)[b]	(48)[b]	(48)[b]	(48)[b]
Histiocytic sarcoma	1 (2%)	1 (2%)		
Leukemia mononuclear	27 (56%)	21 (44%)	24 (50%)	24 (50%)
Mesothelioma malignant		3 (6%)	1 (2%)	2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	48	48	48
Total primary neoplasms	145	159	199	166
Total animals with benign neoplasms	47	47	46	44
Total benign neoplasms	99	109	131	108
Total animals with malignant neoplasms	35	35	39	35
Total malignant neoplasms	46	50	68	58
Total animals with metastatic neoplasms		2	3	1
Total metastatic neoplasms		3	4	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	0.5%	1.0%	1.5%
All Organs: Histiocytic Sarcoma				
Overall rate[a]	1/48 (2%)	1/48 (2%)	0/48 (0%)	0/48 (0%)
Adjusted rate[b]	2.7%	2.8%	0.0%	0.0%
Terminal rate[c]	0/15 (0%)	1/17 (6%)	0/19 (0%)	0/15 (0%)
First incidence (days)[d]	620	729 (T)	----	----
Poly-3 test[e]	P=0.178N	P=0.754	P=0.489N	P=0.517N
All Organs: Osteosarcoma				
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	0/48 (0%)
Adjusted rate	0.0%	2.8%	0.0%	0.0%
Terminal rate	0/15 (0%)	1/17 (6%)	0/19 (0%)	0/15 (0%)
First incidence (days)	----	729 (T)	----	----
Poly-3 test	P=0.519N	P=0.495	---	---
All Organs: Mesothelioma				
Overall rate	0/48 (0%)	3/48 (6%)	1/48 (2%)	2/48 (4%)
Adjusted rate	0.0%	8.3%	2.5%	5.8%
Terminal rate	0/15 (0%)	0/17 (0%)	0/19 (0%)	0/15 (0%)
First incidence (days)	----	650	651	618
Poly-3 test	P=0.286	P=0.115	P=0.514	P=0.224
All Organs: Malignant Neoplasms				
Overall rate	35/48 (73%)	35/48 (73%)	39/48 (81%)	35/48 (73%)
Adjusted rate	78.4%	80.1%	85.0%	81.8%
Terminal rate	9/15 (60%)	14/17 (83%)	15/19 (79%)	12/15 (80%)
First incidence (days)	486	466	333	330
Poly-3 test	P=0.292	P=0.530	P=0.284	P=0.445
All Organs: Benign Neoplasms				
Overall rate	47/48 (98%)	47/48 (98%)	46/48 (96%)	44/48 (92%)
Adjusted rate	99.0%	99.4%	99.1%	99.0%
Terminal rate	15/15 (100%)	17/17 (100%)	19/19 (100%)	15/15 (100%)
First incidence (days)	478	466	511	470
Poly-3 test	P=0.707N	P=0.937	P=0.956	P=0.968N
All Organs: Primary Neoplasms				
Overall rate	48/48 (100%)	48/48 (100%)	48/48 (100%)	46/48 (96%)
Adjusted rate	100.0%	100.0%	100.0%	99.8%
Terminal rate	15/15 (100%)	17/17 (100%)	19/19 (100%)	15/15 (100%)
First incidence (days)	478	466	333	330
Poly-3 test	P=0.998N	---	---	P=1.000N
Testes: Adenoma, Interstitial Cell				
Overall rate	37/48 (77%)	39/48 (81%)	35/48 (73%)	34/48 (71%)
Adjusted rate	84.1%	89.4%	80.5%	82.0%
Terminal rate	12/15 (80%)	17/17 (100%)	18/19 (95%)	13/15 (87%)
First incidence (days)	486	466	511	470
Poly-3 test	P=0.284N	P=0.311	P=0.427N	P=0.515N
Testes: Adenoma, Bilateral/Interstitial Cell				
Overall rate	27/48 (56%)	25/48 (52%)	28/48 (58%)	23/48 (48%)
Adjusted rate	65.4%	62.6%	65.4%	61.4%
Terminal rate	11/15 (73%)	14/17 (82%)	15/19 (79%)	12/15 (80%)
First incidence (days)	542	466	511	488
Poly-3 test	P=0.429N	P=0.486N	P=0.594	P=0.444N

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Preputial Gland: Squamous Cell Papilloma				
Overall rate	0/48 (0%)	1/48 (2%)	3/48 (6%)	0/48 (0%)
Adjusted rate	0.0%	2.8%	7.7%	0.0%
Terminal rate	0/15 (0%)	1/17 (6%)	3/19 (16%)	0/15 (0%)
First incidence (days)	----	729 (T)	729 (T)	----
Poly-3 test	P=0.368	P=0.495	P=0.129	---
Preputial Gland: Squamous Cell Carcinoma				
Overall rate	4/48 (8%)	2/48 (4%)	0/48 (0%)	1/48 (2%)
Adjusted rate	10.7%	5.5%	0.0%	2.9%
Terminal rate	1/15 (7%)	1/17 (6%)	0/19 (0%)	0/15 (0%)
First incidence (days)	612	563	----	714
Poly-3 test	P=0.041N	P=0.350N	P=0.054N	P=0.206N
Preputial Gland: Adenoma or Carcinoma				
Overall rate	7/48 (15%)	4/48 (8%)	5/48 (10%)	2/48 (4%)
Adjusted rate	18.1%	11.0%	12.1%	5.8%
Terminal rate	2/15 (13%)	3/17 (18%)	0/19 (0%)	1/15 (7%)
First incidence (days)	547	619	333	638
Poly-3 test	P=0.088N	P=0.295N	P=0.331N	P=0.104N
Preputial Gland: Carcinoma				
Overall rate	2/48 (4%)	0/48 (0%)	3/48 (6%)	1/48 (2%)
Adjusted rate	5.4%	0.0%	7.4%	2.9%
Terminal rate	0/15 (0%)	0/17 (0%)	0/19 (0%)	1/15 (7%)
First incidence (days)	632	----	333	729 (T)
Poly-3 test	P=0.551	P=0.245N	P=0.540	P=0.531N
Preputial Gland: Adenoma				
Overall rate	5/48 (10%)	4/48 (8%)	2/48 (4%)	1/48 (2%)
Adjusted rate	13.1%	11.0%	5.0%	2.9%
Terminal rate	2/15 (13%)	3/17 (18%)	0/19 (0%)	0/15 (0%)
First incidence (days)	547	619	619	638
Poly-3 test	P=0.047N	P=0.531N	P=0.197N	P=0.122N
Lung: Alveolar/Bronchiolar Adenoma				
Overall rate	0/48 (0%)	5/48 (10%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0.0%	13.8%	0.0%	2.9%
Terminal rate	0/15 (0%)	3/17 (18%)	0/19 (0%)	1/15 (7%)
First incidence (days)	----	650	----	729 (T)
Poly-3 test	P=0.441N	P=0.028	---	P=0.485
Skin: Fibroma				
Overall rate	4/48 (8%)	2/48 (4%)	3/48 (6%)	1/48 (2%)
Adjusted rate	10.7%	5.5%	7.6%	2.9%
Terminal rate	3/15 (20%)	1/17 (6%)	2/19 (11%)	0/15 (0%)
First incidence (days)	478	624	613	638
Poly-3 test	P=0.171N	P=0.351N	P=0.469N	P=0.202N
Mammary Gland: Fibroadenoma				
Overall rate	1/44 (2%)	4/46 (9%)	3/48 (6%)	1/41 (2%)
Adjusted rate	2.9%	11.5%	7.7%	3.3%
Terminal rate	1/14 (7%)	4/17 (24%)	3/19 (16%)	0/13 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	541
Poly-3 test	P=0.552N	P=0.180	P=0.353	P=0.732

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Adrenal Medulla: Pheochromocytoma Benign				
Overall rate	3/48 (6%)	0/47 (0%)	2/48 (4%)	4/48 (8%)
Adjusted rate	8.1%	0.0%	5.1%	11.5%
Terminal rate	1/15 (7%)	0/17 (0%)	2/19 (11%)	3/15 (20%)
First incidence (days)	668	----	729 (T)	541
Poly-3 test	P=0.277	P=0.125N	P=0.474N	P=0.465
Adrenal Medulla: Pheochromocytoma Malignant, Bilateral				
Overall rate	4/48 (8%)	2/47 (4%)	1/48 (2%)	1/48 (2%)
Adjusted rate	10.8%	5.7%	2.6%	2.9%
Terminal rate	0/15 (0%)	2/17 (12%)	1/19 (5%)	1/15 (7%)
First incidence (days)	665	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.075N	P=0.360N	P=0.161N	P=0.203N
Adrenal Medulla: Pheochromocytoma Malignant				
Overall rate	7/48 (15%)	12/47 (26%)	15/48 (31%)	8/48 (17%)
Adjusted rate	18.6%	32.2%	36.6%	22.9%
Terminal rate	0/15 (0%)	6/17 (35%)	5/19 (26%)	4/15 (27%)
First incidence (days)	665	466	626	634
Poly-3 test	P=0.276	P=0.133	P=0.058	P=0.433
Thyroid Gland: Carcinoma, C-Cell				
Overall rate	1/48 (2%)	1/47 (2%)	3/48 (6%)	0/48 (0%)
Adjusted rate	2.7%	2.8%	7.6%	0.0%
Terminal rate	1/15 (7%)	1/17 (6%)	2/19 (11%)	0/15 (0%)
First incidence (days)	729 (T)	729 (T)	680	----
Poly-3 test	P=0.544N	P=0.753	P=0.329	P=0.515N
Thyroid Gland: Adenoma, C-Cell				
Overall rate	2/48 (4%)	7/47 (15%)	3/48 (6%)	3/48 (6%)
Adjusted rate	5.4%	18.7%	7.7%	8.6%
Terminal rate	1/15 (7%)	1/17 (6%)	3/19 (16%)	1/15 (7%)
First incidence (days)	647	534	729 (T)	566
Poly-3 test	P=0.520N	P=0.077	P=0.526	P=0.475
Pituitary Gland: Adenoma, Pars Distalis				
Overall rate	30/48 (63%)	30/48 (63%)	27/47 (57%)	22/48 (46%)
Adjusted rate	69.4%	70.1%	63.5%	56.1%
Terminal rate	11/15 (73%)	10/17 (59%)	12/19 (63%)	8/15 (53%)
First incidence (days)	523	489	520	474
Poly-3 test	P=0.088N	P=0.572	P=0.355N	P=0.140N
Islets, Pancreatic: Adenoma				
Overall rate	3/48 (6%)	4/48 (8%)	5/48 (10%)	4/48 (8%)
Adjusted rate	8.2%	11.0%	12.6%	11.5%
Terminal rate	2/15 (13%)	2/17 (12%)	3/19 (16%)	3/15 (20%)
First incidence (days)	711	651	613	566
Poly-3 test	P=0.344	P=0.493	P=0.396	P=0.467
Proximal Colon: Carcinoma				
Overall rate	0/44 (0%)	0/44 (0%)	4/46 (9%)	4/41 (10%)
Adjusted rate	0.0%	0.0%	10.3%	11.7%
Terminal rate	0/15 (0%)	0/17 (0%)	3/19 (16%)	1/15 (7%)
First incidence (days)	----	----	676	474
Poly-3 test	P=0.006	---	P=0.073	P=0.055

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Proximal Colon: Adenoma				
Overall rate	0/44 (0%)	0/44 (0%)	7/46 (15%)	10/41 (24%)
Adjusted rate	0.0%	0.0%	17.9%	28.6%
Terminal rate	0/15 (0%)	0/17 (0%)	5/19 (26%)	3/15 (20%)
First incidence (days)	----	----	651	501
Poly-3 test	P<0.001	---	P=0.011	P<0.001
Intestine Large: All Adenomas				
Overall rate	0/47 (0%)	0/48 (0%)	26/48 (54%)	23/48 (48%)
Adjusted rate	0.0%	0.0%	63.2%	59.8%
Terminal rate	0/15 (0%)	0/17 (0%)	16/19 (84%)	10/15 (67%)
First incidence (days)	----	----	597	488
Poly-3 test	P<0.001	---	P=0.001	P<0.001
Intestine Large: All Carcinomas				
Overall rate	0/47 (0%)	0/48 (0%)	10/48 (21%)	14/48 (29%)
Adjusted rate	0.0%	0.0%	24.9%	36.4%
Terminal rate	0/15 (0%)	0/17 (0%)	5/19 (26%)	4/15 (27%)
First incidence (days)	----	----	619	444
Poly-3 test	P<0.001	---	P=0.001	P<0.001
Intestine Large: All Adenomas or Carcinomas				
Overall rate	0/47 (0%)	0/48 (0%)	28/48 (58%)	31/48 (65%)
Adjusted rate	0.0%	0.0%	66.9%	74.2%
Terminal rate	0/15 (0%)	0/17 (0%)	16/19 (84%)	12/15 (80%)
First incidence (days)	----	----	597	444
Poly-3 test	P<0.001	---	P<0.001	P<0.001
Intestine Large, Transverse Colon: Adenoma				
Overall rate	0/47 (0%)	0/47 (0%)	6/47 (13%)	3/47 (6%)
Adjusted rate	0.0%	0.0%	15.5%	8.8%
Terminal rate	0/15 (0%)	0/17 (0%)	3/19 (16%)	2/15 (13%)
First incidence (days)	----	----	676	634
Poly-3 test	P=0.011	---	P=0.018	P=0.107
Intestine Large, Ascending Colon: Carcinoma				
Overall rate	0/47 (0%)	0/47 (0%)	4/48 (8%)	8/46 (17%)
Adjusted rate	0.0%	0.0%	10.1%	22.4%
Terminal rate	0/15 (0%)	0/17 (0%)	1/19 (5%)	2/15 (13%)
First incidence (days)	----	----	619	444
Poly-3 test	P<0.001	---	P=0.073	P=0.003
Intestine Large, Ascending Colon: Adenoma				
Overall rate	0/47 (0%)	0/47 (0%)	19/48 (40%)	8/46 (17%)
Adjusted rate	0.0%	0.0%	47.3%	23.6%
Terminal rate	0/15 (0%)	0/17 (0%)	13/19 (68%)	5/15 (33%)
First incidence (days)	----	----	641	638
Poly-3 test	P<0.001	---	P<0.001	P=0.002
Intestine Large, Cecum: Adenoma				
Overall rate	0/46 (0%)	0/45 (0%)	8/48 (17%)	8/48 (17%)
Adjusted rate	0.0%	0.0%	19.9%	22.9%
Terminal rate	0/15 (0%)	0/16 (0%)	4/19 (21%)	5/15 (33%)
First incidence (days)	----	----	597	634
Poly-3 test	P<0.001	---	P=0.006	P=0.003

TABLE A2
Statistical Analysis of Neoplasms in Male Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	8/48 (17%)	1/48 (2%)	0/48 (0%)	1/48 (2%)
Adjusted rate	21.3%	2.8%	0.0%	2.9%
Terminal rate	5/15 (33%)	1/17 (6%)	0/19 (0%)	0/15 (0%)
First incidence (days)	535	729 (T)	----	683
Poly-3 test	P<0.001N	P=0.017N	P=0.002N	P=0.020N
Liver: Hepatocellular Adenoma				
Overall rate	5/48 (10%)	1/48 (2%)	0/48 (0%)	0/48 (0%)
Adjusted rate	13.6%	2.8%	0.0%	0.0%
Terminal rate	4/15 (27%)	1/17 (6%)	0/19 (0%)	0/15 (0%)
First incidence (days)	724	729 (T)	----	----
Poly-3 test	P=0.002N	P=0.103N	P=0.024N	P=0.035N
Liver: Hepatocellular Carcinoma				
Overall rate	3/48 (6%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	8.0%	0.0%	0.0%	2.9%
Terminal rate	1/15 (7%)	0/17 (0%)	0/19 (0%)	0/15 (0%)
First incidence (days)	535	----	----	683
Poly-3 test	P=0.121N	P=0.125N	P=0.110N	P=0.337N

[a] Number of neoplasm-bearing animals over number of animals examined.
[b] Poly K incidence; estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at terminal kill.
[d] Time to first lesion in days. T indicates terminal sacrifice.
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by N.

TABLE A3a
Historical Incidence of Cecum and Colon/Rectum Neoplasms in NCTR Control Male F344/N Rats

Study (Report Date)	Route of Administration	Incidence in Controls	
		Cecum Adenoma or Carcinoma	Colon/Rectum Adenoma or Carcinoma
Doxylamine (April 1991)	Diet	0/48	0/48
Fumonisin B_1 (March 1999)	Diet	0/48	0/48
Gentian Violet (November 1988)	Diet	0/162	0/162
Leucomalachite Green (June 2001)	Diet		0/48
Pyrilamine (July 1991)	Diet	0/44	0/44
Sulfamethazine (February 1988)	Diet	0/175	0/175
Triprolidine (June 1991)	Diet	0/42	0/42
Total (%)		0/519 (0.0%)	0/567 (0.0%)
Range		0.0%	0.0%

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract[a]

	0%	0.5%	1.0%	1.5%
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund sacrifice	28	26	23	26
Natural deaths	2	5	2	4
Survivors				
Moribund sacrifice	3		4	3
Terminal sacrifice	15	17	19	15
Animals examined microscopically	48	48	48	48
Alimentary System				
Esophagus	(48)	(48)	(48)	(48)
Inflammation	1 (2%)	1 (2%)	1 (2%)	
Ulcer	1 (2%)	1 (2%)	1 (2%)	
Mucosa, hyperplasia	1 (2%)			
Intestine large, ascending colon	(47)	(47)	(48)	(46)
Hyperplasia				2 (4%)
Inflammation			1 (2%)	2 (4%)
Lymphoid tissue, hyperplasia				1 (2%)
Mucosa, hyperplasia		30 (64%)	38 (79%)	32 (70%)
Intestine large, cecum	(46)	(45)	(48)	(48)
Dilatation	1 (2%)		8 (17%)	17 (35%)
Hemorrhage				1 (2%)
Hyperplasia	1 (2%)			
Inflammation	1 (2%)			2 (4%)
Ulcer				1 (2%)
Lymphoid tissue, hyperplasia		2 (4%)	3 (6%)	1 (2%)
Mucosa, hyperplasia		13 (29%)	24 (50%)	25 (52%)
Intestine large, colon	(0)	(1)	(3)	(5)
Inflammation			1 (33%)	
Ulcer			1 (33%)	
Mucosa, hyperplasia		1 (100%)	1 (33%)	4 (80%)
Intestine large, descending colon	(47)	(46)	(46)	(47)
Inflammation			2 (4%)	
Lymphoid tissue, hyperplasia		1 (2%)		1 (2%)
Mucosa, hyperplasia		17 (37%)	31 (67%)	30 (64%)
Intestine large, rectum	(47)	(47)	(48)	(48)
Mucosa, hyperplasia		1 (2%)	1 (2%)	4 (8%)
Intestine large, transverse colon	(47)	(47)	(47)	(47)
Hyperplasia				1 (2%)
Inflammation			1 (2%)	
Lymphoid tissue, hyperplasia		1 (2%)	2 (4%)	
Mucosa, hyperplasia		30 (64%)	42 (89%)	34 (72%)
Intestine small	(0)	(1)	(0)	(0)
Intestine small, duodenum	(48)	(46)	(48)	(48)
Mucosa, hyperplasia	1 (2%)	10 (22%)	3 (6%)	6 (13%)
Intestine small, ileum	(45)	(45)	(48)	(48)
Hyperplasia	1 (2%)			
Inflammation	1 (2%)		1 (2%)	
Lymphoid tissue, hyperplasia		1 (2%)		2 (4%)
Mucosa, hyperplasia		3 (7%)	3 (6%)	2 (4%)
Intestine small, jejunum	(45)	(44)	(46)	(46)
Inflammation				1 (2%)
Lymphatic, dilatation			1 (2%)	
Lymphoid tissue, hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Mucosa, hyperplasia		1 (2%)	2 (4%)	3 (7%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Alimentary System (continued)				
Liver	(48)	(48)	(48)	(48)
Angiectasis			1 (2%)	1 (2%)
Basophilic focus			2 (4%)	
Basophilic focus, multiple		1 (2%)	1 (2%)	1 (2%)
Cyst multilocular	1 (2%)			1 (2%)
Degeneration, cystic	9 (19%)	6 (13%)	2 (4%)	
Eosinophilic focus	4 (8%)			1 (2%)
Eosinophilic focus, multiple	1 (2%)		1 (2%)	
Granuloma	2 (4%)	5 (10%)	6 (13%)	10 (21%)
Hematopoietic cell proliferation		1 (2%)	1 (2%)	1 (2%)
Hemorrhage			1 (2%)	
Hepatodiaphragmatic nodule	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Infiltration cellular, lymphocyte		1 (2%)	4 (8%)	3 (6%)
Infiltration cellular, polymorphonuclear				1 (2%)
Necrosis, coagulative	1 (2%)	2 (4%)		2 (4%)
Regeneration				1 (2%)
Tension lipidosis				1 (2%)
Vacuolization cytoplasmic	12 (25%)	14 (29%)	6 (13%)	1 (2%)
Artery, media, hypertrophy		1 (2%)		
Bile duct, hyperplasia	15 (31%)	16 (33%)	9 (19%)	5 (10%)
Caudate lobe, developmental malformation			1 (2%)	
Centrilobular, degeneration		1 (2%)	2 (4%)	
Centrilobular, necrosis	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hepatocyte, periportal hypertrophy				1 (2%)
Left lateral lobe, developmental malformation	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Median lobe, developmental malformation				1 (2%)
Periductular, fibrosis		1 (2%)		
Right lateral lobe, developmental malformation				1 (2%)
Mesentery	(10)	(10)	(4)	(5)
Accessory spleen			1 (25%)	
Ectopic tissue				1 (20%)
Hemorrhage	1 (10%)			
Inflammation			1 (25%)	1 (20%)
Polyarteritis		1 (10%)		1 (20%)
Thrombosis	1 (10%)			1 (20%)
Fat, necrosis	7 (70%)	6 (60%)	2 (50%)	2 (40%)
Oral Mucosa	(2)	(1)	(2)	(1)
Keratin Cyst			1 (50%)	
Epithelium, hyperplasia	1 (50%)			
Pancreas	(48)	(48)	(48)	(48)
Infiltration cellular, lymphocyte				1 (2%)
Polyarteritis	3 (6%)		3 (6%)	
Acinar cell, atrophy	12 (25%)	17 (35%)	19 (40%)	24 (505)
Proximal colon	(44)	(44)	(46)	(41)
Hemorrhage				1 (2%)
Hyperplasia, lymphoid				1 (2%)
Inflammation	1 (2%)	4 (9%)	3 (7%)	1 (2%)
Ulcer		2 (5%)		
Mucosa, hyperplasia		29 (66%)	36 (78%)	32 (78%)
Salivary glands	(48)	(48)	(48)	(48)
Acinar cell, atrophy		1 (2%)		
Sublingual gland, infiltration cellular		1 (2%)		
Stomach, Forestomach	(48)	(47)	(48)	(48)
Edema	1 (2%)	4 (9%)	1 (2%)	
Hyperplasia	10 (21%)	13 (28%)	9 (19%)	2 (4%)
Inflammation	11 (23%)	9 (19%)	5 (10%)	3 (6%)
Perforation		1 (2%)		
Ulcer	3 (6%)	3 (6%)	3 (65)	1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Alimentary System (continued)				
Stomach, glandular	(48)	(47)	(48)	(48)
Diverticulum		1 (2%)		
Edema	1 (2%)	1 (2%)		1 (2%)
Erosion	1 (2%)			
Inflammation	1 (2%)	3 (6%)	3 (6%)	
Mineralization	1 (2%)			
Ulcer		1 (2%)		
Mucosa, hyperplasia	1 (2%)	12 (26%)	7 (15%)	11 (23%)
Mucosa, necrosis, focal	2 (4%)		1 (2%)	
Cardiovascular System				
Blood vessel	(48)	(48)	(48)	(48)
Heart	(48)	(48)	(48)	(48)
Cardiomyopathy	34 (71%)	39 (81%)	38 (79%)	32 (67%)
Dilatation			1 (2%)	
Atrium, dilatation			1 (2%)	
Atrium, thrombus	6 (13%)	9 (19%)	10 (21%)	4 (8%)
Atrium, myocardium, degeneration		1 (2%)		
Endocrine System				
Adrenal cortex	(48)	(48)	(48)	(48)
Accessory adrenal cortical nodule		1 (2%)	1 (2%)	
Angiectasis	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Atrophy	1 (2%)	2 (4%)	1 (2%)	
Hyperplasia, focal	2 (4%)	1 (2%)	2 (4%)	3 (6%)
Hypertrophy, focal	3 (6%)		8 (17%)	2 (4%)
Necrosis, coagulative		1 (2%)		
Thrombus				1 (2%)
Vacuolization cytoplasmic	24 (50%)	22 (46%)	15 (31%)	14 (29%)
Adrenal Medulla	(48)	(47)	(48)	(48)
Angiectasis	3 (6%)	2 (4%)	2 (4%)	3 (6%)
Hyperplasia, focal	10 (21%)	4 (9%)	3 (6%)	6 (13%)
Islets, pancreatic	(48)	(48)	(48)	(48)
Hyperplasia		1 (2%)	1 (2%)	
Parathyroid gland	(47)	(48)	(46)	(48)
Hyperplasia, focal				1 (2%)
Pituitary gland	(48)	(48)	(47)	(48)
Angiectasis		2 (4%)	2 (4%)	5 (10%)
Hemorrhage		1 (2%)		1 (2%)
Pars distalis, cyst	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Pars distalis, hyperplasia	5 (10%)		4 (9%)	5 10%)
Pars intermedia, cyst	1 (2%)	1 (2%)		
Thyroid gland	(48)	(47)	(48)	(48)
Ultimobranchial cyst		1 (2%)		
C-cell, hyperplasia	8 (17%)	7 (15%)	6 (13%)	6 (13%)
Follicular cell, hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)
General Body System				
Tissue NOS	(0)	(0)	(1)	(2)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Genital System				
Epididymis	(48)	(48)	(48)	(48)
Atrophy		1 (2%)	1 (2%)	
Exfoliated germ cell	37 (77%)	27 (56%)	36 (75%)	23 (48%)
Granuloma sperm			2 (4%)	
Hypospermia	28 (58%)	25 (52%)	27 (56%)	26 (54%)
Inflammation	1 (2%)			1 (2%)
Preputial gland	(48)	(48)	(48)	(48)
Atrophy		1 (2%)		1 (2%)
Inflammation	38 (79%)	41 (85%)	39 (81%)	37 (77%)
Duct, ectasia	6 (13%)	13 (27%)	13 (27%)	9 (19%)
Duct, hyperplasia		1 (2%)	1 (2%)	
Prostate	(48)	(48)	(48)	(48)
Atrophy		1 (2%)		
Inflammation	34 (71%)	32 (67%)	36 (75%)	27 (56%)
Seminal vesicle	(48)	(48)	(48)	(48)
Atrophy	4 (8%)	11 (23%)	8 (17%)	6 (13%)
Decreased secretory fluid	7 (15%)	8 (17%)	9 (19%)	9 (19%)
Testes	(48)	(48)	(48)	(48)
Granuloma sperm	1 (2%)			
Inflammation	1 (2%)			
Polyarteritis	1 (2%)		1 (2%)	
Interstitial cell, hyperplasia	3 (6%)	2 (4%)	2 (4%)	4 (8%)
Seminiferous tubule, atrophy	17 (35%)	11 (23%)	17 (35%)	8 (17%)
Hematopoietic System				
Bone marrow	(48)	(48)	(48)	(48)
Atrophy	8 (17%)	3 (6%)	4 (8%)	2 (4%)
Hyperplasia	7 (15%)	8 (17%)	4 (8%)	5 (10%)
Myeloid cell, hyperplasia		1 (2%)	1 (2%)	1 (2%)
Lymph node	(24)	(13)	(19)	(17)
Lumbar, degeneration, cystic	4 (17%)	4 (31%)	2 (11%)	1 (6%)
Lumbar, hyperplasia, lymphoid		1 (8%)		1 (6%)
Lumbar, infiltration cellular, plasma cell		1 (8%)		
Mediastinal, degeneration, cystic	1 (4%)		1 (5%)	
Mediastinal, hemorrhage	1 (4%)	2 (15%)	1 (5%)	1 (6%)
Mediastinal , pigmentation		1 (8%)		
Mediastinal, medulla sinus, dilatation			1 (5%)	
Medulla, pancreatic sinus, dilatation	1 (4%)		2 (11%)	
Medulla, renal sinus, dilatation		1 (8%)		
Medulla, sinus, dilatation	1 (4%)			
Pancreatic, degeneration, cystic			1 (5%)	1 (6%)
Pancreatic, hyperplasia, lymphoid	1 (4%)	1 (8%)	3 (16%)	2 (12%)
Pancreatic, infiltration cellular, plasma cell	1 (4%)			
Pancreatic, pigmentation			1 (5%)	
Renal, degeneration, cystic	2 (8%)	1 (8%)	1 (5%)	2 (12%)
Renal, hemorrhage			1 (5%)	
Renal, hyperplasia, lymphoid		2 (15%)		
Renal, infiltration cellular, plasma cell		1 (8%)		
Renal, pigmentation			1 (5%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Hematopoietic System (continued)				
Lymph node, mandibular	(48)	(48)	(48)	(48)
Atrophy, lymphocyte	1 (2%)	1 (2%)		
Degeneration, cystic	11 (23%)	9 (19%)	5 (10%)	5 (10%)
Hematopoietic cell proliferation	1 (2%)			
Hemorrhage		1 (2%)		
Hyperplasia, lymphoid	1 (2%)	2 (4%)	3 (6%)	
Infiltration cellular, plasma cell	9 (19%)	14 (29%)	10 (21%)	11 (23%)
Necrosis, lymphoid	1 (2%)			
Medulla, sinus dilatation		1 (2%)		1 (2%)
Lymph node, mesenteric	(47)	(48)	(48)	(48)
Degeneration, cystic	8 (17%)	11 (23%)	42 (88%)	41 (85%)
Fibrosis				1 (2%)
Hemorrhage	2 (4%)	2 (4%)		1 (2%)
Hyperplasia, lymphoid			1 (2%)	4 (8%)
Infiltration cellular, plasma cell	1 (2%)			
Inflammation				1 (2%)
Necrosis, lymphoid	1 (2%)		1 (2%)	
Medulla, sinus, dilatation		4 (8%)	1 (2%)	
Spleen	(48)	(48)	(48)	(48)
Accessory spleen	1 (2%)		1 (2%)	
Atrophy		2 (4%)		
Congestion	1 (2%)	3 (6%)	2 (4%)	
Fibrosis		1 (2%)	1 (2%)	
Hematopoietic cell proliferation	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)		
Hyperplasia, focal	1 (2%)			
Infarct	6 (13%)	9 (19%)	5 (10%)	1 (2%)
Pigmentation	3 (6%)	9 (19%)	3 (6%)	6 (13%)
Thrombus	1 (2%)		1 (2%)	1 (2%)
Capsule, fibrosis			1 (2%)	
Lymphoid follicle, necrosis	1 (2%)			
Red pulp, hyperplasia			1 (2%)	
Thymus	(46)	(47)	(44)	(44)
Atrophy	38 (83%)	42 (89%)	39 (89%)	40 (91%)
Cyst	1 (2%)	2 (4%)		
Ectopic thyroid		1 (2%)		
Hemorrhage				1 (2%)
Epithelial cell, hyperplasia			1 (2%)	
Integumentary System				
Mammary gland	(44)	(46)	(48)	(41)
Galactocele	9 (20%)	11 (24%)	11 (23%)	10 (24%)
Lactation	23 (52%)	19 (41%)	24 (50%)	10 (24%)
Alveolus, hyperplasia	12 (27%)	14 (30%)	12 (25%)	6 (15%)
Skin	(48)	(48)	(48)	(48)
Cyst epithelial inclusion	1 (2%)	1 (2%)		
Inflammation	1 (2%)	1 (2%)	2 (4%)	
Inflammation, granulomatous		1 (2%)		
Epidermis, necrosis	1 (2%)			
Fat, subcutaneous tissue, necrosis			1 (2%)	
Foot, inflammation, chronic				1 (2%)
Subcutaneous tissue, inflammation, focal		1 (2%)		
Tail, hyperkeratosis, multifocal			1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Musculoskeletal System				
Bone	(0)	(2)	(2)	(1)
Cartilage, sternum, degeneration				1 (100%)
Cranium, periosteum, hemorrhage			1 (50%)	
Bone, femur	(48)	(48)	(48)	(48)
Osteopetrosis	1 (2%)			
Skeletal muscle	(2)	(2)	(0)	(2)
Nervous System				
Brain	(0)	(0)	(1)	(0)
Brain, brain stem	(48)	(48)	(48)	(48)
Hemorrhage		1 (2%)	1 (2%)	
Hypothalamus, compression	11 (23%)	10 (21%)	13 (27%)	9 (19%)
Brain, cerebellum	(48)	(48)	(48)	(48)
Compression			1 (2%)	
Hemorrhage			2 (4%)	
Brain, cerebrum	(48)	(48)	(48)	(48)
Hemorrhage				1 (2%)
Hydrocephalus			1 (2%)	1 (2%)
Mineralization, focal			1 (2%)	
Spinal cord	(1)	(1)	(0)	(3)
Hemorrhage				1 (33%)
Respiratory System				
Lung	(48)	(48)	(48)	(48)
Granuloma			1 (2%)	1 (2%)
Hemorrhage	1 (2%)			1 (2%)
Infiltration cellular, lymphocyte		1 (2%)		
Metaplasia, osseous				1 (2%)
Alveolar epithelium, hyperplasia	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Alveolus, infiltration cellular, histiocyte	3 (6%)	3 (6%)	10 (21%)	6 (13%)
Alveolus, infiltration cellular, lymphocyte			1 (2%)	
Alveolus, inflammation	2 (4%)		3 (6%)	2 (4%)
Mediastinum, inflammation			1 (2%)	
Nose	(48)	(48)	(47)	(48)
Foreign body			1 (2%)	
Fungus	1 (2%)		1 (2%)	
Inflammation	4 (8%)	3 (6%)	4 (9%)	1 (2%)
Goblet cell, hyperplasia	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Trachea	(48)	(48)	(48)	(48)
Mucosa, cyst		1 (2%)		
Special Senses System				
Eye	(47)	(46)	(47)	(48)
Cataract	2 (4%)			2 (4%)
Phthisis bulbi				1 (2%)
Retina, degeneration	5 (11%)	2 (4%)		4 (8%)
Sclera, metaplasia, osseous	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Harderian gland	(48)	(48)	(48)	(48)
Atrophy	1 (2%)			
Infiltration cellular, lymphocyte	7 (15%)	11 (23%)	8 (17%)	8 (17%)
Inflammation			1 (2%)	2 (4%)
Lacrimal gland	(0)	(1)	(0)	(0)
Zymbal's gland	(0)	(2)	(1)	(0)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Urinary System				
Kidney	(48)	(48)	(48)	(48)
Hydronephrosis		1 (2%)	1 (2%)	
Nephropathy	48 (100%)	47 (98%)	48 (100%)	45 (94%)
Cortex, cyst	2 (4%)			1 (2%)
Renal tubule, necrosis		1 (2%)		
Urinary bladder	(48)	(48)	(48)	(48)
Dilatation	1 (2%)	5 (10%)	1 (2%)	2 (4%)
Hemorrhage				1 (2%)
Hyperplasia			1 (2%)	
Inflammation			1 (2%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF ALOE VERA WHOLE LEAF EXTRACT

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract[a]

	0%	0.5%	1.0%	1.5%
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund sacrifice	15	15	17	20
Natural deaths	2		4	5
Survivors				
Moribund sacrifice	1	2	2	2
Natural death			1	1
Terminal sacrifice	30	31	24	20
Animals examined microscopically	48	48	48	48
Alimentary System				
Esophagus	(48)	(47)	(48)	(48)
Intestine large, ascending colon	(47)	(48)	(46)	(46)
Adenoma			1 (2%)	5 (11%)
Carcinoma			1 (2%)	1 (2%)
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear			1 (2%)	1 (2%)
Intestine large, cecum	(47)	(48)	(47)	(48)
Adenoma			1 (2%)	5 (10%)
Adenoma, multiple				1 (2%)
Lipoma		1 (2%)		
Intestine large, colon	(0)	(0)	(2)	(1)
Adenoma				1 (100%)
Intestine large, descending colon	(47)	(48)	(46)	(47)
Leukemia mononuclear			1 (2%)	1 (2%)
Intestine large, rectum	(48)	(48)	(47)	(47)
Intestine large, transverse colon	(47)	(48)	(46)	(46)
Carcinoma				1 (2%)
Histiocytic sarcoma			1 (2%)	
Leiomyosarcoma				1 (2%)
Leukemia mononuclear			1 (2%)	
Intestine small, duodenum	(48)	(48)	(48)	(48)
Leiomyoma			1 (2%)	
Intestine small, ileum	(47)	(48)	(43)	(44)
Leiomyosarcoma			1 (2%)	
Leukemia mononuclear		1 (2%)	1 (2%)	
Intestine small, jejunum	(47)	(48)	(45)	(43)
Leukemia mononuclear				1 (2%)
Liver	(48)	(48)	(48)	(48)
Hepatocellular adenoma	1 (2%)			
Histiocytic sarcoma			2 (4%)	
Leukemia mononuclear	9 (19%)	9 (19%)	16 (33%)	16 (33%)
Mesentery	(6)	(9)	(10)	(4)
Histiocytic sarcoma			1 (10%)	
Leukemia mononuclear			2 (20%)	
Oral mucosa	(0)	(1)	(1)	(0)
Squamous cell carcinoma			1 (100%)	
Squamous cell papilloma		1 (100%)		
Pancreas	(48)	(48)	(48)	(48)
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear	2 (4%)		1 (2%)	1 (2%)
Proximal colon	(43)	(45)	(42)	(39)
Adenoma			4 (10%)	5 (13%)
Carcinoma			2 (5%)	4 (10%)
Leukemia mononuclear				1 (3%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Alimentary System (continued)				
Salivary glands	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)		1 (2%)	
Stomach, forestomach	(48)	(48)	(48)	(48)
Leukemia mononuclear			1 (2%)	
Squamous cell papilloma	1 (2%)			1 (2%)
Stomach, glandular	(48)	(48)	(48)	(48)
Leukemia mononuclear			1 (2%)	
Cardiovascular System				
Heart	(48)	(48)	(48)	(48)
Leukemia mononuclear	5 (10%)	2 (4%)	5 (10%)	7 (15%)
Schwannoma malignant	1 (2%)			
Endocrine System				
Adrenal cortex	(48)	(48)	(48)	(48)
Adenoma		1 (2%)		1 (2%)
Leukemia mononuclear		1 (2%)	2 (4%)	4 (8%)
Adrenal medulla	(47)	(46)	(48)	(47)
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear	4 (9%)		1 (2%)	5 (11%)
Pheochromocytoma malignant			2 (4%)	
Bilateral, pheochromocytoma malignant				1 (2%)
Islets, pancreatic	(48)	(48)	(47)	(48)
Leukemia mononuclear			1 (2%)	
Parathyroid gland	(47)	(45)	(46)	(47)
Adenoma			1 (2%)	1 (2%)
Pituitary gland	(48)	(48)	(48)	(47)
Leukemia mononuclear	1 (2%)		1 (2%)	2 (4%)
Pars distalis, adenoma	32 (67%)	35 (73%)	30 (63%)	18 (38%)
Pars distalis, carcinoma	1 (2%)	1 (2%)		
Thyroid gland	(48)	(48)	(48)	(47)
Histiocytic sarcoma			1 (2%)	
Bilateral, c-cell, carcinoma			1 (2%)	
C-cell, adenoma	5 (10%)	3 (6%)	1 (2%)	3 (6%)
C-cell, carcinoma	1 (2%)	1 (2%)	3 (6%)	
General Body System				
Tissue NOS	(0)	(0)	(2)	(0)
Mediastinum, histiocytic sarcoma			1 (50%)	
Genital System				
Clitoral gland	(48)	(48)	(48)	(48)
Adenoma	5 (10%)	8 (17%)	3 (6%)	3 (6%)
Adenoma, multiple		1 (2%)		
Carcinoma	3 (6%)	4 (8%)	1 (2%)	2 (4%)
Leukemia mononuclear			1 (2%)	
Ovary	(48)	(48)	(48)	(48)
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear	3 (6%)		3 (6%)	3 (6%)
Uterus	(48)	(48)	(48)	(48)
Leiomyoma				1 (2%)
Leiomyosarcoma	1 (2%)			
Leukemia mononuclear			1 (2%)	2 (4%)
Polyp stromal	11 (23%)	13 (27%)	12 (25%)	8 (17%)
Endometrium, adenocarcinoma			1 (2%)	1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Genital System (continued)				
Vagina	(4)	(3)	(2)	(4)
Leiomyosarcoma, metastatic, uterus	1 (25%)			
Polyp		1 (33%)		
Hematopoietic System				
Bone marrow	(48)	(48)	(48)	(47)
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear	1 (2%)	1 (2%)		2 (4%)
Lymph node	(9)	(7)	(8)	(9)
Axillary, leukemia mononuclear	1 (11%)		2 (25%)	1 (11%)
Brachial, leukemia mononuclear		1 (14%)		
Lumbar, leukemia mononuclear	3 (33%)	2 (29%)	2 (25%)	2 (22%)
Mediastinal, histiocytic sarcoma			1 (13%)	
Mediastinal, leukemia mononuclear	5 (56%)	1 (14%)	2 (25%)	3 (33%)
Pancreatic, leukemia mononuclear	5 (56%)	4 (57%)	3 (38%)	5 (56%)
Renal, leukemia mononuclear	2 (22%)		1 (13%)	3 (33%)
Thoracic, leukemia mononuclear		1 (14%)		
Lymph node, mandibular	(48)	(47)	(48)	(47)
Leukemia mononuclear	5 (10%)	4 (9%)	6 (13%)	6 (13%)
Lymph node, mesenteric	(46)	(47)	(48)	(47)
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear	7 (15%)	4 (9%)	7 (15%)	9 (19%)
Spleen	(48)	(48)	(48)	(48)
Hemangiosarcoma	1 (2%)			
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear	9 (19%)	12 (25%)	19 (40%)	18 (38%)
Thymus	(46)	(45)	(45)	(44)
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear	3 (7%)		4 (9%)	1 (2%)
Integumentary System				
Mammary gland	(47)	(48)	(48)	(47)
Adenocarcinoma	1 (2%)	4 (8%)		
Fibroadenoma	12 (26%)	15 (31%)	9 (19%)	8 (17%)
Leukemia mononuclear				1 (2%)
Skin	(48)	(48)	(48)	(48)
Basal cell carcinoma	1 (2%)		1 (2%)	
Fibroma			1 (2%)	
Sarcoma	1 (2%)		2 (4%)	
Ear, squamous cell papilloma	1 (2%)			
Head, basal cell carcinoma				1 (2%)
Subcutaneous tissue, fibroma			1 (2%)	
Musculoskeletal System				
Bone	(0)	(2)	(0)	(0)
Bone, femur	(48)	(48)	(48)	(48)
Skeletal muscle	(0)	(3)	(0)	(2)
Nervous System				
Brain, brain stem	(48)	(48)	(48)	(48)
Leukemia mononuclear			1 (2%)	1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Nervous System (continued)				
Brain, cerebellum	(48)	(48)	(48)	(48)
Carcinoma, metastatic, pituitary gland	1 (2%)			
Leukemia mononuclear			2 (4%)	2 (4%)
Meningioma malignant				1 (2%)
Brain, cerebrum	(48)	(48)	(48)	(48)
Astrocytoma malignant			1 (2%)	
Leukemia mononuclear	1 (2%)		2 (4%)	3 (6%)
Meningioma malignant				1 (2%)
Respiratory System				
Lung	(48)	(48)	(48)	(48)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)		
Carcinoma, metastatic, thyroid gland			1 (2%)	
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear	8 (17%)	6 (13%)	12 (25%)	11 (23%)
Nose	(48)	(48)	(48)	(48)
Special Senses System				
Ear	(0)	(0)	(1)	(0)
Eye	(48)	(48)	(46)	(46)
Harderian gland	(48)	(48)	(48)	(48)
Leukemia mononuclear			1 (2%)	
Zymbal's gland	(1)	(0)	(1)	(1)
Carcinoma			1 (100%)	
Squamous cell carcinoma	1 (100%)			1 (100%)
Urinary System				
Kidney	(48)	(48)	(48)	(48)
Leukemia mononuclear			1 (2%)	2 (4%)
Sarcoma				1 (2%)
Urinary bladder	(48)	(48)	(48)	(48)
Leukemia mononuclear			1 (2%)	2 (4%)
Transitional epithelium, papilloma	1 (2%)			
Systemic Lesions				
Multiple organs	(48)[b]	(48)[b]	(48)[b]	(48)[b]
Histiocytic sarcoma			2 (4%)	
Leukemia mononuclear	10 (21%)	12 (25%)	19 (40%)	18 (38%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	45	48	44	39
Total primary neoplasms	92	102	104	95
Total animals with benign neoplasms	41	47	41	33
Total benign neoplasms	70	80	65	61
Total animals with malignant neoplasms	22	21	32	25
Total malignant neoplasms	22	22	39	34
Total animals with metastatic neoplasms	2		1	
Total metastatic neoplasms	2		1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Statistical Analysis of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	0.5%	1.0%	1.5%
All Organs: Histiocytic Sarcoma				
Overall rate[a]	0/48 (0%)	0/48 (0%)	2/48 (4%)	0/48 (0%)
Adjusted rate[b]	0.0%	0.0%	5.2%	0.0%
Terminal rate[c]	0/30 (0%)	0/31 (0%)	1/24 (4%)	0/20 (0%)
First incidence (days)[d]	----	----	679	----
Poly-3 test[e]	P=0.334	---	P=0.222	---
All Organs: Malignant Neoplasms				
Overall rate	22/48 (46%)	21/48 (44%)	32/48 (67%)	25/48 (52%)
Adjusted rate	50.4%	46.9%	74.0%	61.6%
Terminal rate	12/30 (40%)	11/31 (36%)	16/24 (67%)	12/20 (60%)
First incidence (days)	478	464	428	446
Poly-3 test	P=0.032	P=0.452N	P=0.016	P=0.201
All Organs: Benign Neoplasms				
Overall rate	41/48 (85%)	47/48 (98%)	41/48 (85%)	33/48 (69%)
Adjusted rate	90.4%	98.6%	93.7%	81.3%
Terminal rate	28/30 (93%)	31/31 (100%)	24/24 (100%)	18/20 (90%)
First incidence (days)	396	348	407	476
Poly-3 test	P=0.047N	P=0.077	P=0.420	P=0.157N
All Organs: Primary Neoplasms				
Overall rate	45/48 (94%)	48/48 (100%)	44/48 (92%)	39/48 (81%)
Adjusted rate	95.7%	100.0%	96.0%	91.5%
Terminal rate	28/30 (93%)	31/31 (100%)	24/24 (100%)	20/20 (100%)
First incidence (days)	396	348	407	446
Poly-3 test	P=0.111N	P=0.233	P=0.696	P=0.326N
Uterus: Polyp Stromal				
Overall rate	11/48 (23%)	13/48 (27%)	12/48 (25%)	8/48 (17%)
Adjusted rate	25.9%	30.5%	29.9%	21.4%
Terminal rate	8/30 (27%)	10/31 (32%)	7/24 (29%)	4/20 (20%)
First incidence (days)	396	464	407	516
Poly-3 test	P=0.387N	P=0.405	P=0.436	P=0.420N
Clitoral Gland: Carcinoma or Adenoma				
Overall rate	8/48 (17%)	13/48 (27%)	4/48 (8%)	5/48 (10%)
Adjusted rate	19.4%	30.1%	10.5%	13.5%
Terminal rate	7/30 (23%)	8/31 (26%)	3/24 (13%)	3/20 (15%)
First incidence (days)	676	464	679	642
Poly-3 test	P=0.105N	P=0.189	P=0.211N	P=0.348N
Clitoral Gland: Carcinoma				
Overall rate	3/48 (6%)	4/48 (8%)	1/48 (2%)	2/48 (4%)
Adjusted rate	7.3%	9.3%	2.6%	5.4%
Terminal rate	2/30 (7%)	1/31 (3%)	1/24 (4%)	1/20 (5%)
First incidence (days)	676	464	729 (T)	642
Poly-3 test	P=0.286N	P=0.525	P=0.333N	P=0.552N
Clitoral Gland: Adenoma				
Overall rate	5/48 (10%)	9/48 (19%)	3/48 (6%)	3/48 (6%)
Adjusted rate	12.2%	21.7%	7.8%	8.2%
Terminal rate	5/30 (17%)	7/31 (23%)	2/24 (8%)	2/20 (10%)
First incidence (days)	729 (T)	690	679	684
Poly-3 test	P=0.173N	P=0.194	P=0.394N	P=0.419N

TABLE B2
Statistical Analysis of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Mammary Gland: Fibroadenoma				
Overall rate	12/47 (26%)	15/48 (31%)	9/48 (19%)	8/47 (17%)
Adjusted rate	28.9%	34.7%	22.9%	22.0%
Terminal rate	9/30 (30%)	10/31 (32%)	5/24 (21%)	5/20 (25%)
First incidence (days)	669	515	547	575
Poly-3 test	P=0.178N	P=0.367	P=0.359N	P=0.331N
Mammary Gland: Adenocarcinoma				
Overall rate	1/47 (2%)	4/48 (8%)	0/48 (0%)	0/47 (0%)
Adjusted rate	2.4%	9.6%	0.0%	0.0%
Terminal rate	1/30 (3%)	3/31 (10%)	0/24 (0%)	0/20 (0%)
First incidence (days)	729 (T)	661	----	----
Poly-3 test	P=0.148N	P=0.181	P=0.515N	P=0.529N
Thyroid Gland: Carcinoma or Adenoma, C-Cell				
Overall rate	6/48 (13%)	4/48 (8%)	5/48 (10%)	3/47 (6%)
Adjusted rate	14.6%	9.6%	13.1%	8.5%
Terminal rate	5/30 (17%)	2/31 (7%)	4/24 (17%)	3/20 (15%)
First incidence (days)	680	669	725	729 (T)
Poly-3 test	P=0.306N	P=0.363N	P=0.555N	P=0.319N
Thyroid Gland: Carcinoma, C-Cell				
Overall rate	1/48 (2%)	1/48 (2%)	4/48 (8%)	0/47 (0%)
Adjusted rate	2.4%	2.4%	10.5%	0.0%
Terminal rate	1/30 (3%)	1/31 (3%)	3/24 (13%)	0/20 (0%)
First incidence (days)	729 (T)	729 (T)	725	----
Poly-3 test	P=0.495	P=0.760N	P=0.156	P=0.529N
Thyroid Gland: Adenoma, C-Cell				
Overall rate	5/48 (10%)	3/48 (6%)	1/48 (2%)	3/47 (6%)
Adjusted rate	12.1%	7.2%	2.6%	8.5%
Terminal rate	4/30 (13%)	1/31 (3%)	1/24 (4%)	3/20 (15%)
First incidence (days)	680	669	729 (T)	729 (T)
Poly-3 test	P=0.222N	P=0.350N	P=0.119N	P=0.440N
Pituitary Gland: Adenoma, Pars Distalis				
Overall rate	32/48 (67%)	35/48 (73%)	30/48 (63%)	18/47 (38%)
Adjusted rate	72.4%	76.4%	70.9%	48.4%
Terminal rate	22/30 (73%)	23/31 (74%)	18/24 (75%)	12/20 (60%)
First incidence (days)	463	348	469	528
Poly-3 test	P=0.014N	P=0.421	P=0.536N	P=0.018N
Proximal Colon: Carcinoma				
Overall rate	0/43 (0%)	0/45 (0%)	2/42 (5%)	4/39 (10%)
Adjusted rate	0.0%	0.0%	5.7%	11.6%
Terminal rate	0/30 (0%)	0/31 (0%)	2/24 (8%)	2/20 (10%)
First incidence (days)	----	----	729 (T)	679
Poly-3 test	P=0.004	---	P=0.212	P=0.043
Proximal Colon: Adenoma				
Overall rate	0/43 (0%)	0/45 (0%)	4/42 (10%)	5/39 (13%)
Adjusted rate	0.0%	0.0%	11.3%	14.4%
Terminal rate	0/30 (0%)	0/31 (0%)	4/24 (17%)	3/20 (15%)
First incidence (days)	----	----	729 (T)	661
Poly-3 test	P=0.001	---	P=0.046	P=0.019

TABLE B2
Statistical Analysis of Neoplasms in Female Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Intestine Large: All Adenomas				
Overall rate	0/48 (0%)	0/48 (0%)	6/48 (13%)	13/48 (27%)
Adjusted rate	0.0%	0.0%	15.7%	33.8%
Terminal rate	0/30 (0%)	0/31 (0%)	5/24 (21%)	8/20 (40%)
First incidence (days)	----	----	684	476
Poly-3 test	P<0.001	---	P=0.011	P<0.001
Intestine Large: All Carcinomas				
Overall rate	0/48 (0%)	0/48 (0%)	3/48 (6%)	4/48 (8%)
Adjusted rate	0.0%	0.0%	7.9%	10.9%
Terminal rate	0/30 (0%)	0/31 (0%)	3/24 (13%)	2/20 (10%)
First incidence (days)	----	----	729 (T)	679
Poly-3 test	P=0.005	---	P=0.105	P=0.047
Intestine Large: All Adenomas or Carcinomas				
Overall rate	0/48 (0%)	0/48 (0%)	8/48 (17%)	15/48 (31%)
Adjusted rate	0.0%	0.0%	20.9%	38.8%
Terminal rate	0/30 (0%)	0/31 (0%)	7/24 (29%)	9/20 (45%)
First incidence (days)	----	----	684	476
Poly-3 test	P<0.001	---	P=0.002	P<0.001
Intestine Large, Ascending Colon: Adenoma				
Overall rate	0/47 (0%)	0/48 (0%)	1/46 (2%)	5/46 (11%)
Adjusted rate	0.0%	0.0%	2.7%	13.9%
Terminal rate	0/30 (0%)	0/31 (0%)	0/24 (0%)	3/20 (15%)
First incidence (days)	----	----	684	516
Poly-3 test	P=0.002	---	P=0.480	P=0.021
Intestine Large, Cecum: Adenoma				
Overall rate	0/47 (0%)	0/48 (0%)	1/47 (2%)	6/48 (13%)
Adjusted rate	0.0%	0.0%	2.6%	15.7%
Terminal rate	0/30 (0%)	0/31 (0%)	1/24 (4%)	3/20 (15%)
First incidence (days)	----	----	729 (T)	476
Poly-3 test	P<0.001	---	P=0.487	P=0.012

[a] Number of neoplasm-bearing animals over number of animals examined.
[b] Poly K incidence; estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at terminal kill.
[d] Time to first lesion in days. T indicates terminal sacrifice.
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by N.

TABLE B3a
Historical Incidence of Cecum and Colon/Rectum Neoplasms in NCTR Control Female F344/N Rats

Study (Report Date)	Route of Administration	Incidence in Controls	
		Cecum Adenoma or Carcinoma	Colon/Rectum Adenoma or Carcinoma
Doxylamine (April 1991)	Diet	0/48	0/48
Fumonisin B_1 (March 1999)	Diet	0/47	0/47
Gentian Violet (November 1988)	Diet	0/161	0/161
Leucomalachite Green (June 2001)	Diet		0/48
Malachite Green (June 2001)	Diet		0/48
Pyrilamine (July 1991)	Diet	0/48	0/48
Sulfamethazine (February 1988)	Diet	0/178	0/178
Triprolidine (June 1991)	Diet	0/45	0/45
Total (%)		0/527 (0.0%)	0/623 (0.0%)
Range		0%	0.0%

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract[a]

	0%	0.5%	1.0%	1.5%
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund sacrifice	15	15	17	20
Natural deaths	2		4	5
Survivors				
Moribund sacrifice	1	2	2	2
Natural death			1	1
Terminal sacrifice	30	31	24	20
Animals examined microscopically	48	48	48	48
Alimentary System				
Esophagus	(48)	(47)	(48)	(48)
Inflammation	1 (2%)			
Intestine large, ascending colon	(47)	(48)	(46)	(46)
Dilatation			1 (2%)	1 (2%)
Inflammation		1 (2%)	1 (2%)	2 (4%)
Lymphoid tissue, hyperplasia			2 (4%)	2 (4%)
Mucosa, hyperplasia		40 (83%)	35 (76%)	39 (85%)
Intestine large, cecum	(47)	(48)	(47)	(48)
Dilatation			9 (19%)	25 (52%)
Inflammation		1 (2%)	2 (4%)	2 (4%)
Perforation				1 (2%)
Lymphoid tissue, hyperplasia		1 (2%)		2 (4%)
Mucosa, hyperplasia		4 (8%)	17 (36%)	27 (56%)
Intestine large, colon	(0)	(0)	(2)	(1)
Inflammation			1 (50%)	
Necrosis			1 (50%)	
Mucosa, hyperplasia			1 (50%)	
Intestine large, descending colon	(47)	(48)	(46)	(47)
Inflammation			1 (2%)	
Ulcer			1 (2%)	
Mucosa, hyperplasia		17 (35%)	18 (39%)	27 (57%)
Intestine large, rectum	(48)	(48)	(47)	(47)
Polyarteritis		1 (2%)		
Mucosa, hyperplasia				5 (11%)
Intestine large, transverse colon	(47)	(48)	(46)	(46)
Dilatation			1 (2%)	
Inflammation		1 (2%)	1 (2%)	
Lymphoid tissue, hyperplasia		1 (2%)	1 (2%)	
Mucosa, hyperplasia		40 (83%)	33 (72%)	42 (91%)
Intestine small, duodenum	(48)	(48)	(48)	(48)
Inflammation			1 (2%)	1 (2%)
Mucosa, hyperplasia	1 (2%)	4 (8%)	5 (10%)	2 (4%)
Intestine small, ileum	(47)	(48)	(43)	(44)
Inflammation		1 (2%)	1 (2%)	
Lymphoid tissue, hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Mucosa, hyperplasia		2 (4%)	2 (5%)	6 (14%)
Intestine small, jejunum	(47)	(48)	(45)	(43)
Inflammation		2 (4%)		
Ulcer		1 (2%)		
Lymphatic, dilatation		1 (2%)		
Submucosa, fibrosis		1 (2%)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Alimentary System (continued)				
Liver	(48)	(48)	(48)	(48)
Angiectasis			1 (2%)	1 (2%)
Basophilic focus		1 (2%)		1 (2%)
Basophilic focus, multiple	22 (46%)	27 (56%)	12 (25%)	13 (27%)
Cyst multilocular	1 (2%)			
Eosinophilic focus	5 (10%)	7 (15%)	2 (4%)	1 (2%)
Eosinophilic focus, multiple		1 (2%)		1 (2%)
Granuloma	21 (44%)	19 (40%)	20 (42%)	15 (31%)
Hematopoietic cell proliferation	1 (2%)			
Hepatodiaphragmatic nodule	4 (8%)	2 (4%)	4 (8%)	2 (4%)
Infiltration cellular, lymphocyte	1 (2%)	3 (6%)	1 (2%)	3 (6%)
Necrosis, coagulative			2 (4%)	
Regeneration				1 (2%)
Tension lipidosis			2 (4%)	
Vacuolization cytoplasmic	6 (13%)	9 (19%)	4 (8%)	5 (10%)
Bile duct, hyperplasia	9 (19%)	5 (10%)	6 (13%)	2 (4%)
Caudate lobe, developmental malformation			4 (8%)	1 (2%)
Caudate lobe, infarct				1 (2%)
Centrilobular, necrosis				2 (4%)
Left lateral lobe, developmental malformation	4 (8%)	2 (4%)	3 (6%)	1 (2%)
Left lateral lobe, infarct				1 (2%)
Median lobe, developmental malformation				1 (2%)
Periportal, inflammation, chronic			1 (2%)	1 (2%)
Right lateral lobe, developmental malformation			2 (4%)	
Mesentery	(6)	(9)	(10)	(4)
Polyarteritis		1 (11%)	1 (10%)	
Thrombosis		1 (11%)		
Fat, necrosis	6 (100%)	8 (89%)	6 (60%)	4 (100%)
Oral Mucosa	(0)	(1)	(1)	(0)
Pancreas	(48)	(48)	(48)	(48)
Accessory Spleen			1 (2%)	
Polyarteritis		1 (2%)	1 (2%)	
Acinar cell, atrophy	10 (21%)	11 (23%)	17 (35%)	17 (35%)
Proximal colon	(43)	(45)	(42)	(39)
Dilatation			1 (2%)	
Foreign body				1 (3%)
Inflammation		2 (4%)	11 (26%)	8 (21%)
Ulcer		1 (2%)	2 (5%)	1 (3%)
Mucosa, hyperplasia		30 (67%)	33 (79%)	32 (82%)
Salivary glands	(48)	(48)	(48)	(48)
Acinar cell, atrophy	1 (2%)			
Stomach, forestomach	(48)	(48)	(48)	(48)
Edema		2 (4%)	2 (4%)	1 (2%)
Hyperplasia	1 (2%)	7 (15%)	10 (21%)	9 (19%)
Inflammation			4 (8%)	3 (6%)
Stomach, glandular	(48)	(48)	(48)	(48)
Edema		1 (2%)		1 (2%)
Inflammation			1 (2%)	1 (2%)
Ulcer				1 (2%)
Mucosa, hyperplasia		1 (2%)	3 (6%)	3 (6%)
Mucosa, necrosis, focal		1 (2%)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Cardiovascular System				
Heart	(48)	(48)	(48)	(48)
Cardiomyopathy	35 (73%)	40 (83%)	32 (67%)	33 (69%)
Atrium, thrombus			1 (2%)	1 (2%)
Endocrine System				
Adrenal cortex	(48)	(48)	(48)	(48)
Accessory adrenal cortical nodule			1 (2%)	
Angiectasis	31 (65%)	25 (52%)	20 (42%)	19 (40%)
Atrophy				1 (2%)
Fibrosis, focal	1 (2%)			
Hemorrhage				1 (2%)
Hyperplasia, focal		2 (4%)		2 (4%)
Hypertrophy	1 (2%)			
Hypertrophy, focal	2 (4%)	2 (4%)	6 (13%)	1 (2%)
Necrosis, coagulative				1 (2%)
Vacuolization cytoplasmic	6 (13%)	7 (15%)	5 (10%)	6 (13%)
Adrenal medulla	(47)	(46)	(48)	(47)
Angiectasis	2 (4%)	6 (13%)	1 (2%)	6 (13%)
Hyperplasia, focal		1 (2%)	1 (2%)	1 (2%)
Islets, pancreatic	(48)	(48)	(47)	(48)
Parathyroid gland	(47)	(45)	(46)	(47)
Hyperplasia, focal	1 (2%)			
Pituitary gland	(48)	(48)	(48)	(47)
Angiectasis	2 (4%)	1 (2%)		1 (2%)
Pigmentation				1 (2%)
Pars distalis, cyst	1 (2%)	5 (10%)	4 (8%)	6 (13%)
Pars distalis, hyperplasia	3 (6%)	3 (6%)	2 (4%)	4 (9%)
Pars intermedia, cyst			1 (2%)	1 (2%)
Pars nervosa, cyst				1 (2%)
Thyroid gland	(48)	(48)	(48)	(47)
C-cell, hyperplasia	15 (31%)	13 (27%)	8 (17%)	3 (6%)
Follicular cell, hyperplasia				1 (2%)
General Body System				
Tissue NOS	(0)	(0)	(2)	(0)
Fat, necrosis			1 (50%)	
Genital System				
Clitoral gland	(48)	(48)	(48)	(48)
Atrophy				1 (2%)
Hyperplasia			1 (2%)	
Inflammation	33 (69%)	26 (54%)	30 (63%)	30 (63%)
Duct, ectasia	12 (25%)	15 (31%)	8 (17%)	8 (17%)
Duct, hyperplasia	2 (4%)		2 (4%)	5 (10%)
Ovary	(48)	(48)	(48)	(48)
Atrophy	47 (98%)	44 (92%)	42 (88%)	47 (98%)
Cyst	2 (4%)	4 (8%)	6 (13%)	4 (8%)
Uterus	(48)	(48)	(48)	(48)
Hyperplasia, focal	2 (4%)	1 (2%)	2 (4%)	5 (10%)
Hypoplasia	1 (2%)			
Inflammation	1 (2%)	2 (4%)		2 (4%)
Adventitia, inflammation			1 (2%)	
Bilateral, horn, dilatation				1 (2%)
Cervix, mucocyte, metaplasia	1 (2%)	1 (2%)		
Cervix, muscularis, hyperplasia		1 (2%)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Genital System (continued)				
Uterus (continued)				
Cervix, muscularis, hypertrophy	1 (2%)	1 (2%)	1 (2%)	
Endometrium, hyperplasia, cystic	10 (21%)	16 (33%)	8 (17%)	12 (25%)
Horn, dilatation	1 (2%)	2 (4%)	1 (2%)	
Vagina	(4)	(3)	(2)	(4)
Dilatation	1 (25%)	1 (33%)	1 (50%)	
Inflammation, suppurative	1 (25%)			3 (75%)
Mucocyte, hyperplasia	3 (75%)	1 (33%)	2 (100%)	4 (100%)
Hematopoietic System				
Bone marrow	(48)	(48)	(48)	(47)
Atrophy		2 (4%)	1 (2%)	
Hyperplasia	1 (2%)	4 (8%)	3 (6%)	1 (2%)
Proliferation				1 (2%)
Myeloid cell, hyperplasia			3 (6%)	1 (2%)
Lymph node	(9)	(7)	(8)	(9)
Degeneration, cystic			2 (25%)	
Lumbar, degeneration, cystic	2 (22%)			
Lumbar, hyperplasia, lymphoid	1 (11%)			
Mediastinal, hemorrhage		1 (14%)	2 (25%)	
Mediastinal, hyperplasia, lymphoid				1 (11%)
Mediastinal, polyarteritis		1 (14%)		
Mediastinal, medulla sinus, dilatation		1 (14%)		
Medulla, pancreatic sinus, dilatation				1 (11%)
Pancreatic, degeneration, cystic				1 (11%)
Pancreatic, hemorrhage			1 (13%)	
Pancreatic, hyperplasia, lymphoid		1 (14%)		1 (11%)
Lymph node, mandibular	(48)	(47)	(48)	(47)
Cyst	2 (4%)	1 (2%)		
Degeneration, cystic	2 (4%)	3 (6%)	3 (6%)	4 (9%)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Infiltration cellular, plasma cell	7 (15%)	9 (19%)	9 (19%)	4 (9%)
Medulla, sinus, dilatation	2 (4%)			2 (4%)
Lymph node, mesenteric	(46)	(47)	(48)	(47)
Amyloid deposition			1 (2%)	1 (2%)
Atrophy, lymphocyte				4 (9%)
Degeneration, cystic		16 (34%)	40 (83%)	43 (91%)
Fibrosis				1 (2%)
Hemorrhage	3 (7%)	3 (6%)	1 (2%)	
Hyperplasia, lymphoid		2 (4%)	2 (4%)	3 (6%)
Medulla, sinus, dilatation	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Spleen	(48)	(48)	(48)	(48)
Accessory spleen	2 (4%)	1 (2%)		
Atrophy	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Fibrosis	1 (2%)			
Hematopoietic cell proliferation	4 (8%)	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, focal	1 (2%)	5 (10%)	1 (2%)	1 (2%)
Infarct	1 (2%)	2 (4%)		3 6%)
Pigmentation	7 (15%)	6 (13%)	5 (10%)	6 (13%)
Thrombus	2 (4%)			
Red pulp, hyperplasia		3 (6%)		
Thymus	(46)	(45)	(45)	(44)
Atrophy	43 (93%)	44 (98%)	40 (89%)	42 (95%)
Cyst		1 (2%)		1 (2%)
Hemorrhage	1 (2%)			

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Integumentary System				
Mammary gland	(47)	(48)	(48)	(47)
Galactocele	3 (6%)	1 (2%)	3 (6%)	5 (11%)
Lactation	27 (57%)	28 (58%)	24 (50%)	23 (49%)
Alveolus, hyperplasia	27 (57%)	38 (79%)	28 (58%)	22 (47%)
Alveolus, hypertrophy	1 (2%)			
Skin	(48)	(48)	(48)	(48)
Cyst epithelial inclusion			1 (2%)	
Inflammation		1 (2%)		
Epidermis, hyperplasia	1 (2%)			1 (2%)
Epidermis, necrosis	1 (2%)	1 (2%)		1 (2%)
Foot, hyperkeratosis	1 (2%)			
Musculoskeletal System				
Bone	(0)	(2)	(0)	(0)
Cervical, vertebra, fracture		2 (100%)		
Bone, femur	(48)	(48)	(48)	(48)
Fibrous osteodystrophy		1 (2%)		
Osteopetrosis	8 (17%)	3 (6%)	4 (8%)	5 (10%)
Skeletal muscle	(0)	(3)	(0)	(2)
Polyarteritis		1 (33%)		
Diaphragm, inflammation		1 (33%)		
Nervous System				
Brain, brain stem	(48)	(48)	(48)	(48)
Hypothalamus, compression	13 (27%)	14 (29%)	8 (17%)	8 (17%)
Brain, cerebellum	(48)	(48)	(48)	(48)
Brain, cerebrum	(48)	(48)	(48)	(48)
Hemorrhage			1 (2%)	
Hydrocephalus	1 (2%)	1 (2%)		
Respiratory System				
Lung	(48)	(48)	(48)	(48)
Granuloma	6 (13%)	4 (8%)	4 (8%)	3 (6%)
Hemorrhage		2 (4%)	1 (2%)	
Alveolar epithelium, hyperplasia	4 (8%)	2 (4%)	1 (2%)	
Alveolus, infiltration cellular, histiocyte	13 (27%)	8 (17%)	9 (19%)	11 (23%)
Alveolus, inflammation	3 (6%)	4 (8%)	2 (4%)	
Artery, mineralization			1 (2%)	
Nose	(48)	(48)	(48)	(48)
Inflammation	1 (2%)	6 (13%)	8 (17%)	2 (4%)
Osteopetrosis	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Goblet cell, hyperplasia		1 (2%)	1 (2%)	
Special Senses System				
Ear	(0)	(0)	(1)	(0)
Canal, external ear, inflammation			1 (100%)	
Eye	(48)	(48)	(46)	(46)
Cataract		2 (4%)	1 (2%)	1 (2%)
Hemorrhage		1 (2%)		
Phthisis bulbi		1 (2%)		
Retina, degeneration	4 (8%)	8 (17%)	3 (7%)	3 (7%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	0.5%	1.0%	1.5%
Special Senses System (continued)				
Harderian gland	(48)	(48)	(48)	(48)
Hyperplasia			1 (2%)	
Infiltration cellular, lymphocyte	24 (50%)	21 (44%)	18 (38%)	17 (35%)
Inflammation	1 (2%)	1 (2%)		
Zymbal's gland	(1)	(0)	(1)	(1)
Urinary System				
Kidney	(48)	(48)	(48)	(48)
Hydronephrosis	1 (2%)			
Infarct	2 (4%)		2 (4%)	
Mineralization	40 (83%)	35 (73%)	40 (83%)	35 (73%)
Nephropathy	47 (98%)	45 (94%)	40 (83%)	40 (83%)
Cortex, cyst				1 (2%)
Cortex, inflammation, chronic			1 (2%)	
Epithelium, pelvis, hyperplasia			1 (2%)	
Renal tubule, pigmentation				1 (2%)
Urinary Bladder	(48)	(48)	(48)	(48)
Dilatation	1 (2%)			
Inflammation				1 (2%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF ALOE VERA WHOLE LEAF EXTRACT

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract[a]

	0%	1%	2%	3%
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund sacrifice	16	16	23	15
Natural death		2	4	4
Survivors				
Natural death	1	1		1
Terminal sacrifice	31	28	21	28
Animals examined microscopically	48	47	48	48
Alimentary System				
Esophagus	(48)	(46)	(47)	(47)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Gallbladder	(46)	(43)	(45)	(43)
Lymphoma malignant			1 (2%)	1 (2%)
Intestine large, ascending colon	(47)	(44)	(45)	(42)
Intestine large, cecum	(47)	(44)	(44)	(43)
Lymphoma malignant			1 (2%)	
Intestine large, descending colon	(47)	(44)	(45)	(43)
Intestine large, transverse colon	(47)	(44)	(45)	(43)
Intestine small, duodenum	(47)	(44)	(44)	(44)
Fibrous histiocytoma				1 (2%)
Intestine small, ileum	(47)	(44)	(45)	(43)
Lymphoma malignant		1 (2%)	1 (2%)	
Intestine small, jejunum	(47)	(44)	(43)	(43)
Carcinoma		1 (2%)		
Lymphoma malignant	1 (2%)	3 (7%)		1 (2%)
Liver	(47)	(46)	(47)	(46)
Cholangiocarcinoma			1 (2%)	1 (2%)
Fibrous histiocytoma				1 (2%)
Hemangiosarcoma	3 (6%)			2 (4%)
Hepatoblastoma		1 (2%)		
Hepatocellular adenoma	3 (6%)	6 (13%)	4 (9%)	2 (4%)
Hepatocellular adenoma, multiple	3 (6%)			
Hepatocellular carcinoma	8 (17%)	13 (28%)	8 (17%)	9 (20%)
Hepatocellular carcinoma, multiple	1 (2%)			1 (2%)
Hepatocholangiocarcinoma		1 (2%)	1 (2%)	
Histiocytic sarcoma			1 (2%)	1 (2%)
Lymphoma malignant		1 (2%)	4 (9%)	1 (2%)
Mesentery	(0)	(4)	(2)	(4)
Fibrous histiocytoma				1 (25%)
Pancreas	(47)	(46)	(47)	(45)
Cholangiocarcinoma, metastatic, liver			1 (2%)	
Fibrous histiocytoma				1 (2%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)	1 (2%)	
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant			4 (9%)	1 (2%)
Salivary glands	(48)	(45)	(46)	(45)
Lymphoma malignant	1 (2%)		1 (2%)	
Stomach, forestomach	(47)	(45)	(45)	(44)
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma		1 (2%)		2 (5%)
Stomach, glandular	(47)	(44)	(45)	(45)
Fibrous histiocytoma				1 (2%)
Lymphoma malignant			1 (2%)	
Serosa, hepatcholangiocarcinoma, metastatic, liver			1 (2%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Cardiovascular System				
Blood vessel	(48)	(47)	(47)	(47)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Lymphoma malignant			1 (2%)	
Heart	(48)	(47)	(47)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Cholagniocarcinoma, metastatic, liver			1 (2%)	
Fibrosarcoma, metastatic, skin				1 (2%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Lymphoma malignant			1 (2%)	
Endocrine System				
Adrenal cortex	(48)	(44)	(46)	(45)
Cholangiocarcinoma, metastatic, liver			1 (2%)	
Fibrous histiocytoma				1 (2%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Lymphoma malignant	1 (2%)	1 (2%)	2 (4%)	
Subcapsular, adenoma	1 (2%)	3 (7%)	2 (4%)	
Adrenal medulla	(47)	(44)	(46)	(44)
Cholangiocarcinoma, metastatic, liver			1 (2%)	
Lymphoma malignant			1 (2%)	
Pheochromocytoma benign			3 (7%)	1 (2%)
Pheochromocytoma malignant				1 (2%)
Islets, pancreatic	(47)	(46)	(46)	(45)
Lymphoma malignant			1 (2%)	
Parathyroid gland	(44)	(46)	(42)	(41)
Pituitary gland	(48)	(45)	(45)	(45)
Thyroid gland	(47)	(46)	(47)	(45)
Lymphoma malignant			1 (2%)	
Follicular cell, adenoma			1 (2%)	
Follicular cell, carcinoma			1 (2%)	
General Body System				
Tissue NOS	(1)	(1)	(3)	(0)
Cholangiocarcinoma, metastatic, liver			1 (33%)	
Hepatocholangiocarcinoma, metastatic, liver			1 (33%)	
Mediastinum, fibrosarcoma, metastatic, skin	1 (100%)			
Mediastinum, hepatocholangiocarcinoma, metastatic, liver		1 (100%)		
Genital System				
Coagulating gland	(1)	(1)	(0)	(0)
Epididymis	(48)	(44)	(46)	(45)
Fibrous histiocytoma				1 (2%)
Hemangioma			1 (2%)	
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)	1 (2%)	
Lymphoma malignant	1 (2%)	1 (2%)	2 (4%)	
Penis	(0)	(1)	(0)	(0)
Preputial gland	(47)	(44)	(46)	(44)
Fibrosarcoma	1 (2%)			
Fibrous histiocytoma	1 (2%)			
Lymphoma malignant			1 (2%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Genital System (continued)				
Prostate	(47)	(44)	(46)	(44)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Lymphoma malignant	1 (2%)		2 (4%)	
Seminal vesicle	(48)	(44)	(47)	(44)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Lymphoma malignant			2 (4%)	
Testes	(47)	(44)	(45)	(44)
Hemangioma				1 (2%)
Hematopoietic System				
Bone marrow	(47)	(45)	(47)	(45)
Histiocytic sarcoma			1 (2%)	
Lymph node	(11)	(5)	(9)	(7)
Axillary, lymphoma malignant	2 (18%)	1 (20%)	1 (11%)	
Illiac, lymphoma malignant	1 (9%)			
Inguinal, lymphoma malignant			1 (11%)	
Lumbar, histiocytic sarcoma			1 (11%)	
Lumbar, lymphoma malignant		2 (40%)	5 (56%)	1 (14%)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung	1 (9%)			
Mediastinal, fibrous histiocytoma				1 (14%)
Mediastinal, histiocytic sarcoma			1 (11%)	
Mediastinal, lymphoma malignant		1 (20%)	4 (44%)	1 (14%)
Pancreatic, lymphoma malignant	1 (9%)	2 (40%)	2 (22%)	1 (14%)
Renal, fibrosarcoma, metastatic, skin	1 (9%)			
Renal, histiocytic sarcoma			1 (11%)	
Renal, lymphoma malignant	1 (9%)	2 (40%)	4 (44%)	2 (29%)
Lymph node, mandibular	(47)	(44)	(45)	(44)
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant	1 (2%)	2 (5%)	5 (11%)	
Lymph node, mesenteric	(48)	(45)	(45)	(43)
Hemangiosarcoma	1 (2%)		1 (2%)	
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant	3 (6%)	5 (11%)	5 (11%)	2 (5%)
Spleen	(48)	(45)	(46)	(44)
Fibrous histiocytoma				1 (2%)
Hemangiosarcoma		1 (2%)		
Histiocytic sarcoma			1 (2%	
Lymphoma malignant	4 (8%)	3 (7%)	5 (11%)	2 (5%)
Thymus	(41)	(39)	(37)	(40)
Hepatocholangiocarcinoma, metastatic, liver		1 (3%)	1 (3%)	
Lymphoma malignant	2 (5%)		3 (8%)	1 (3%)
Integumentary System				
Skin	(48)	(47)	(46)	(48)
Carcinoma				1 (2%)
Fibroma	3 (6%)		2 (4%)	1 (2%)
Fibrosarcoma	11 (23%)	10 (21%)	13 (28%)	9 (19%)
Fibrous histiocytoma	2 (4%)			1 (2%)
Hemangioma	1 (2%)			
Hemangiosarcoma		1 (2%)		
Lymphoma malignant			2 (4%)	
Melanoma malignant			1 (2%)	
Neoplasm NOS			1 (2%)	
Sarcoma			1 (2%)	1 (2%)
Schwannoma malignant	1 (2%)	2 (4%)	3 (7%)	1 (2%)
Squamous cell papilloma				2 (4%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Musculoskeletal System				
Skeletal muscle	(1)	(1)	(3)	(0)
Cholangiocarcinoma, metastatic, liver			1 (33%)	
Hepatocholangiocarcinoma, metastatic, liver		1 (100%)	1 (33%)	
Lymphoma malignant			1 (33%)	
Intercostal, fibrosarcoma, metastatic, skin	1 (100%)			
Nervous System				
Brain, cerebrum	(47)	(46)	(46)	(43)
Respiratory System				
Lung	(48)	(47)	(46)	(47)
Alveolar/bronchiolar adenoma	3 (6%)	1 (2%)	4 (9%)	3 (6%)
Alveolar/bronchiolar carcinoma	3 (6%)	1 (2%)	3 (7%)	1 (2%)
Carcinoma, metastatic, skin				1 (2%)
Cholangiocarcinoma, metastatic, liver			1 (2%)	
Fibrosarcoma, metastatic, skin	1 (2%)		1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver		3 (6%)		
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)	1 (2%)	
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant	1 (2%)		2 (4%)	
Nose	(48)	(47)	(47)	(47)
Trachea	(48)	(47)	(46)	(45)
Lymphoma malignant			1 (2%)	
Special Senses System				
Eye	(47)	(43)	(44)	(43)
Harderian gland	(48)	(44)	(46)	(44)
Adenoma	3 (6%)	2 (5%)	2 (4%)	2 (5%)
Carcinoma	1 (2%)			
Lymphoma malignant	1 (2%)			
Urinary System				
Kidney	(48)	(45)	(46)	(45)
Cholangiocarcinoma, metastatic, liver			1 (2%)	
Fibrous histiocytoma				1 (2%)
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Capsule, hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Renal tubule, carcinoma				1 (2%)
Transitional epithelium, carcinoma				1 (2%)
Urethra	(1)	(0)	(0)	(1)
Urinary bladder	(48)	(45)	(45)	(44)
Lymphoma malignant	1 (2%)	1 (2%)	1 (2%)	
Systemic Lesions				
Multiple organs	(48)[b]	(47)[b]	(48)[b]	(48)[b]
Histiocytic sarcoma			1 (2%)	1 (2%)
Lymphoma malignant	4 (8%)	6 (13%)	5 (10%)	2 (4%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Neoplasm Summary				
Total animals with primary neoplasms[c]	39	33	40	33
Total primary neoplasms	54	50	59	58
Total animals with benign neoplasms	15	11	16	12
Total benign neoplasms	17	13	19	14
Total animals with malignant neoplasms	30	28	32	27
Total malignant neoplasms	37	37	39	44
Total animals with metastatic neoplasms	2	4	4	2
Total metastatic neoplasms	5	15	20	3
Total animals with neoplasms uncertain – benign or malignant			1	
Total uncertain neoplasms			1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Statistical Analysis of Neoplasms in Male Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	1%	2%	3%
All Organs: Lymphoma Malignant				
Overall rate[a]	4/48 (8%)	6/47 (13%)	5/48 (10%)	2/48 (4%)
Adjusted rate[b]	10.3%	15.1%	13.3%	5.2%
Terminal rate[c]	3/31 (10%)	4/28 (14%)	1/21 (5%)	2/28 (7%)
First incidence (days)[d]	717	692	658	728 (T)
Poly-3 test[e]	P=0.261N	P=0.382	P=0.478	P=0.343N
All Organs: Histiocytic Sarcoma				
Overall rate	0/48 (0%)	0/47 (0%)	1/48 (2%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	2.7%	2.6%
Terminal rate	0/31 (0%)	0/28 (0%)	0/21 (0%)	0/28 (0%)
First incidence (days)	----	----	658	701
Poly-3 test	P=0.169	---	P=0.490	P=0.498
All Organs: Malignant Neoplasms				
Overall rate	30/48 (63%)	28/47 (60%)	32/48 (67%)	27/48 (56%)
Adjusted rate	62.5%	62.6%	68.7%	58.6%
Terminal rate	13/31 (42%)	13/28 (46%)	7/21 (33%)	11/28 (39%)
First incidence (days)	337	489	357	357
Poly-3 test	P=0.464N	P=0.580	P=0.338	P=0.431N
All Organs: Benign Neoplasms				
Overall rate	15/48 (31%)	11/47 (23%)	16/48 (33%)	12/48 (25%)
Adjusted rate	37.7%	27.2%	41.8%	29.9%
Terminal rate	13/31 (42%)	7/28 (25%)	11/21 (52%)	6/28 (21%)
First incidence (days)	420	598	408	547
Poly-3 test	P=0.419N	P=0.218N	P=0.446	P=0.305N
All Organs: Primary Neoplasms				
Overall rate	39/48 (81%)	33/47 (70%)	40/48 (83%)	33/48 (69%)
Adjusted rate	81.3%	73.4%	84.4%	71.3%
Terminal rate	22/31 (71%)	17/28 (61%)	14/21 (67%)	16/28 (57%)
First incidence (days)	337	489	357	357
Poly-3 test	P=0.267N	P=0.255N	P=0.447	P=0.183N
Liver: Hemangiosarcoma				
Overall rate	3/47 (6%)	0/46 (0%)	0/47 (0%)	2/46 (4%)
Adjusted rate	7.9%	0.0%	0.0%	5.4%
Terminal rate	3/31 (10%)	0/28 (0%)	0/21 (0%)	2/28 (7%)
First incidence (days)	728 (T)	----	----	728 (T)
Poly-3 test	P=0.363N	P=0.114N	P=0.126N	P=0.507N
Liver: Hepatocellular Carcinoma				
Overall rate	9/47 (19%)	13/46 (28%)	8/47 (17%)	10/46 (22%)
Adjusted rate	23.0%	31.7%	21.2%	25.5%
Terminal rate	7/31 (23%)	6/28 (21%)	3/21 (14%)	5/28 (18%)
First incidence (days)	394	526	610	554
Poly-3 test	P=0.488N	P=0.267	P=0.532N	P=0.504
Liver: Hepatocellular Adenoma				
Overall rate	6/47 (13%)	6/46 (13%)	4/47 (9%)	2/46 (4%)
Adjusted rate	15.4%	15.5%	11.0%	5.4%
Terminal rate	4/31 (13%)	5/28 (18%)	3/21 (14%)	2/28 (7%)
First incidence (days)	420	681	710	728 (T)
Poly-3 test	P=0.086N	P=0.620	P=0.412N	P=0.144N

TABLE C2
Statistical Analysis of Neoplasms in Male Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	14/47 (30%)	18/46 (39%)	11/47 (23%)	11/46 (24%)
Adjusted rate	34.9%	43.7%	29.0%	28.1%
Terminal rate	10/31 (32%)	10/28 (36%)	5/21 (24%)	6/28 (21%)
First incidence (days)	394	526	610	554
Poly-3 test	P=0.162N	P=0.278	P=0.376N	P=0.339N
Adrenal Cortex: Adenoma, Subcapsular				
Overall rate	1/48 (2%)	3/44 (7%)	2/46 (4%)	0/45 (0%)
Adjusted rate	2.6%	8.1%	5.6%	0.0%
Terminal rate	1/31 (3%)	2/28 (7%)	2/21 (10%)	0/28 (0%)
First incidence (days)	728 (T)	692	728 (T)	----
Poly-3 test	P=0.306N	P=0.290	P=0.471	P=0.512N
Adrenal Medulla: Pheochromocytoma Benign				
Overall rate	0/47 (0%)	0/44 (0%)	3/46 (7%)	1/44 (2%)
Adjusted rate	0.0%	0.0%	8.4%	2.8%
Terminal rate	0/31 (0%)	0/28 (0%)	2/21 (10%)	0/27 (0%)
First incidence (days)	----	----	710	701
Poly-3 test	P=0.122	---	P=0.108	P=0.489
Skin: Fibroma				
Overall rate	3/48 (6%)	0/47 (0%)	2/46 (4%)	1/48 (2%)
Adjusted rate	7.8%	0.0%	5.6%	2.6%
Terminal rate	3/31 (10%)	0/28 (0%)	1/21 (5%)	0/28 (0%)
First incidence (days)	728 (T)	----	708	609
Poly-3 test	P=0.302N	P=0.115N	P=0.536N	P=0.305N
Skin: Fibrosarcoma				
Overall rate	11/48 (23%)	10/47 (21%)	13/46 (28%)	9/48 (19%)
Adjusted rate	24.5%	23.5%	31.9%	21.5%
Terminal rate	0/31 (0%)	1/28 (4%)	1/21 (5%)	1/28 (4%)
First incidence (days)	337	489	357	423
Poly-3 test	P=0.528N	P=0.554N	P=0.304	P=0.468N
Skin: Fibroma or Fibrosarcoma				
Overall rate	14/48 (29%)	10/47 (21%)	14/46 (30%)	10/48 (21%)
Adjusted rate	31.2%	23.5%	34.3%	23.6%
Terminal rate	3/31 (10%)	1/28 (4%)	2/21 (10%)	1/28 (4%)
First incidence (days)	337	489	357	423
Poly-3 test	P=0.370N	P=0.285N	P=0.470	P=0.290N
Skin: Schwannoma Malignant				
Overall rate	1/48 (2%)	2/47 (4%)	3/46 (7%)	1/48 (2%)
Adjusted rate	2.5%	5.0%	8.1%	2.6%
Terminal rate	0/31 (0%)	1/28 (4%)	0/21 (0%)	0/28 (0%)
First incidence (days)	574	598	441	518
Poly-3 test	P=0.489	P=0.505	P=0.284	P=0.759
Lung: Alveolar/Bronchiolar Adenoma				
Overall rate	3/48 (6%)	1/47 (2%)	4/46 (9%)	3/47 (6%)
Adjusted rate	7.8%	2.5%	10.9%	7.9%
Terminal rate	3/31 (10%)	1/28 (4%)	2/21 (10%)	2/28 (7%)
First incidence (days)	728 (T)	728 (T)	408	681
Poly-3 test	P=0.372	P=0.299N	P=0.470	P=0.657

TABLE C2
Statistical Analysis of Neoplasms in Male Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Lung: Alveolar/Bronchiolar Carcinoma				
Overall rate	3/48 (6%)	1/47 (2%)	3/46 (7%)	1/47 (2%)
Adjusted rate	7.6%	2.5%	8.2%	2.6%
Terminal rate	2/31 (7%)	0/28 (0%)	1/21 (5%)	1/28 (4%)
First incidence (days)	491	692	584	728 (T)
Poly-3 test	P=0.338N	P=0.304N	P=0.627	P=0.319N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma				
Overall rate	6/48 (13%)	2/47 (4%)	7/46 (15%)	4/47 (9%)
Adjusted rate	15.2%	5.1%	18.7%	10.5%
Terminal rate	5/31 (16%)	1/28 (4%)	3/21 (14%)	3/28 (11%)
First incidence (days)	491	692	408	681
Poly-3 test	P=0.535N	P=0.129N	P=0.460	P=0.389N
Lung: Hepatocellular Carcinoma				
Overall rate	0/48 (0%)	3/47 (6%)	0/46 (0%)	0/47 (0%)
Adjusted rate	0.0%	7.5%	0.0%	0.0%
Terminal rate	0/31 (0%)	0/28 (0%)	0/21 (0%)	0/28 (0%)
First incidence (days)	----	631	----	----
Poly-3 test	P=0.306N	P=0.124	---	---
Harderian Gland: Adenoma				
Overall rate	3/48 (6%)	2/44 (5%)	2/46 (4%)	2/44 (5%)
Adjusted rate	7.8%	5.3%	5.5%	5.6%
Terminal rate	3/31 (10%)	0/28 (0%)	0/21 (0%)	1/28 (4%)
First incidence (days)	728 (T)	598	624	695
Poly-3 test	P=0.418N	P=0.512N	P=0.531N	P=0.533N

[a] Number of neoplasm-bearing animals over number of animals examined.
[b] Poly K incidence; estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at terminal kill.
[d] Time to first lesion in days. T indicates terminal sacrifice.
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by N.

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract[a]

	0%	1%	2%	3%
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund sacrifice	16	16	23	15
Natural death		2	4	4
Survivors				
Natural death	1	1		1
Terminal sacrifice	31	28	21	28
Animals examined microscopically	48	47	48	48
Alimentary System				
Esophagus	(48)	(46)	(47)	(47)
Gallbladder	(46)	(43)	(45)	(43)
Intestine large, ascending colon	(47)	(44)	(45)	(42)
Goblet cell, hyperplasia	2 (4%)	16 (36%)	20 (44%)	19 (45)
Intestine large, cecum	(47)	(44)	(44)	(43)
Hyperplasia, lymphoid	2 (4%)			
Goblet cell, hyperplasia				1 (2%)
Intestine large, descending colon	(47)	(44)	(45)	(43)
Goblet cell, hyperplasia		7 (16%)	12 (27%)	17 (40%)
Intestine large, transverse colon	(47)	(44)	(45)	(43)
Goblet cell, hyperplasia	4 (9%)	14 (32%)	21 (47%)	22 (51%)
Intestine small, duodenum	(47)	(44)	(44)	(44)
Intestine small, ileum	(47)	(44)	(45)	(43)
Hyperplasia, lymphoid	1 (2%)			
Intestine small, jejunum	(47)	(44)	(43)	(43)
Hyperplasia, lymphoid			1 (2%)	1 (2%)
Liver	(47)	(46)	(47)	(46)
Angiectasis				1 (2%)
Basophilic focus	8 (17%)	6 (13%)	5 (11%)	5 (11%)
Basophilic focus, multiple				1 (2%)
Congestion	1 (2%)			
Cyst	1 (2%)			1 (2%)
Deformity	1 (2%)			
Degeneration, cystic	1 (2%)			
Eosinophilic focus	1 (2%)			
Fatty change	1 (2%)			
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)	2 (4%)	4 (9%)
Infiltration cellular, polymorphonuclear				1 (2%)
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	3 (7%)
Karyomegaly				1 (2%)
Mixed cell focus	3 (6%)			
Necrosis		1 (2%)	1 (2%)	1 (2%)
Tension lipidosis		7 (15%)	4 (9%)	2 (4%)
Thrombus		1 (2%)		
Vacuolization cytoplasmic	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Mesentery	(0)	(4)	(2)	(4)
Fat, necrosis		4 (100%)	2 (100%)	3 (75%)
Pancreas	(47)	(46)	(47)	(45)
Infiltration cellular, lymphocyte	2 (4%)	3 (7%)	1 (2%)	4 (9%)
Vacuolization cytoplasmic	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Acinus, degeneration	3 (6%)	2 (4%)		
Duct, dilatation			1 (2%)	
Salivary glands	(48)	(45)	(46)	(45)
Infiltration cellular, lymphocyte	39 (81%)	31 (69%)	23 (50%)	29 (64%)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Alimentary System (continued)				
Stomach, forestomach	(47)	(45)	(45)	(44)
Epithelium, hyperplasia	1 (2%)			
Stomach, glandular	(47)	(44)	(45)	(45)
Inflammation, chronic active			1 (2%)	1 (2%)
Epithelium, hyperplasia	1 (2%)	2 (5%)	3 (7%)	2 (4%)
Cardiovascular System				
Blood vessel	(48)	(47)	(47)	(47)
Heart	(48)	(47)	(47)	(47)
Cardiomyopathy	2 (4%)		2 (4%)	1 (2%)
Inflammation, chronic active	1 (2%)			
Endocrine System				
Adrenal cortex	(48)	(44)	(46)	(45)
Accessory adrenal cortical nodule	3 (6%)			1 (2%)
Cyst				1 (2%)
Hematopoietic cell proliferation			1 (2%)	
Hypertrophy		1 (2%)		1 (2%)
Mineralization	1 (2%)			
Subcapsular, hyperplasia	43 (90%)	34 (77%)	32 (70%)	35 (78%)
Adrenal Medulla	(47)	(44)	(46)	(44)
Hyperplasia	4 (9%)	2 (5%)	5 (11%)	4 (9%)
Islets, pancreatic	(47)	(46)	(46)	(45)
Hyperplasia	9 (19%)	3 (7%)	1 (2%)	2 (4%)
Parathyroid gland	(44)	(46)	(42)	(41)
Cyst	1 (2%)			
Infiltration cellular, lymphocyte			1 (2%)	
Pituitary gland	(48)	(45)	(45)	(45)
Pars distalis, cyst		1 (2%)	4 (9%)	3 (7%)
Pars intermedia, hyperplasia	1 (2%)			
Thyroid gland	(47)	(46)	(47)	(45)
Cyst		1 (2%)		
Ectopic thymus	1 (2%)			
Infiltration cellular, lymphocyte	1 (2%)	3 (7%)	1 (2%)	3 (7%)
Inflammation, chronic active			2 (4%)	
Follicle, degeneration	6 (13%)	6 (13%)	3 (6%)	4 (9%)
Follicular cell, hyperplasia		1 (2%)		
General Body System				
Tissue NOS	(1)	(1)	(3)	(0)
Cyst			1 (33%)	
Genital System				
Coagulating gland	(1)	(1)	(0)	(0)
Lumen, dilatation	1 (100%)			
Epididymis	(48)	(44)	(46)	(45)
Atrophy			1 (2%)	
Degeneration	1 (2%)			
Fibrosis			1 (2%)	
Hypospermia	1 (2%)	2 (5%)	2 (4%)	
Infiltration cellular, lymphocyte		2 (5%)		
Inflammation, chronic active				1 (2%)
Spermatocele		1 (2%)	1 (2%)	
Serosa, hyperplasia				1 (2%)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Genital System (continued)				
Penis	(0)	(1)	(0)	(0)
Inflammation, chronic active		1 (100%)		
Necrosis		1 (100%)		
Ulcer		1 (100%)		
Preputial gland	(47)	(44)	(46)	(45)
Cyst	4 (9%)	6 (14%)	5 (11%)	4 (9%)
Degeneration	15 (32%)	11 (25%)	10 (22%)	17 (38%)
Infiltration cellular, lymphocyte	3 (6%)	4 (9%)	4 (9%)	5 (11%)
Inflammation, suppurative	2 (4%)	6 (14%)	6 (13%)	1 (2%)
Inflammation, chronic active	1 (2%)	4 (9%)	4 (9%)	
Bilateral, cyst			1 (2%)	
Duct, ectasia	3 (6%)	6 (14%)	3 (7%)	
Fat, necrosis	1 (2%)		1 (2%)	1 (2%)
Prostate	(47)	(44)	(46)	(44)
Infiltration cellular, lymphocyte	7 (15%)	7 (16%)	6 (13%)	6 (14%)
Inflammation, suppurative			1 (2%)	
Inflammation, chronic active				1 (2%)
Seminal vesicle	(48)	(44)	(47)	(44)
Atrophy			1 (2%)	
Infiltration cellular, lymphocyte		1 (2%)		
Inflammation, suppurative			1 (2%)	
Inflammation, chronic active	1 (2%)			
Lumen, dilatation	5 (10%)	4 (9%)	4 (9%)	5 (11%)
Testes	(47)	(44)	(45)	(44)
Interstitial cell, hyperplasia		1 (2%)		1 (2%)
Seminiferous tubule, degeneration	4 (9%)	4 (9%)	11 (24%)	3 (7%)
Hematopoietic System				
Bone marrow	(47)	(45)	(47)	(45)
Hyperplasia	8 (17%)	9 (20%)	13 (28%)	7 (16%)
Lymph node	(11)	(5)	(9)	(7)
Axillary, hyperplasia, lymphoid	3 (27%)		2 (22%)	1 (14%)
Axillary, infiltration cellular, plasma cell			1 (11%)	
Inguinal, hyperplasia, lymphoid	2 (18%)		3 (33%)	1 (14%)
Inguinal, infiltration cellular, plasma cell		1 (20%)	2 (22%)	1 (14%)
Lumbar, erythrophagocytosis				1 (14%)
Lumbar, hyperplasia, lymphoid		1 (20%)		1 (14%)
Lumbar, infiltration cellular, plasma cell				1 (14%)
Mediastinal, hyperplasia, lymphoid	2 (18%)			
Mediastinal, infiltration cellular, plasma cell	1 (9%)			
Pancreatic, erythrophagocytosis				1 (14%)
Pancreatic, infiltration cellular, plasma cell				1 (14%)
Renal, erythrophagocytosis				1 (14%)
Renal, hyperplasia, lymphoid	1 (9%)		1 (11%)	
Renal, infiltration cellular, plasma cell			1 (11%)	1 (14%)
Lymph node, mandibular	(47)	(44)	(45)	(44)
Hyperplasia, lymphoid	9 (19%)	5 (11%)	4 (9%)	2 (5%)
Infiltration cellular, plasma cell		1 (2%)	1 (2%)	1 (2%)
Pigmentation		1 (2%)		
Lymph node, mesenteric	(48)	(45)	(45)	(43)
Angiectasis	5 (10%)	1 (2%)	2 (4%)	1 (2%)
Hematopoietic cell proliferation	1 (2%)			1 (2%)
Hemorrhage	10 (21%)	13 (29%)	10 (22%)	11 (26%)
Hyperplasia, lymphoid	28 (58 %)	17 (38%)	17 (38%)	23 (52%)
Infiltration cellular, histiocyte			2 (4%)	4 (9%)
Infiltration cellular, plasma cell				2 (4%)
Infiltration cellular, polymorphonuclear		1 (2%)	2 (4%)	1 (2 %)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Hematopoietic System (continued)				
Lymph node, mesenteric (continued)				
Thrombus		1 (2%)		1 (2%)
Sinus, dilatation	6 (13%)	2 (4%)	4 (9%)	2 (5%)
Spleen	(48)	(45)	(46)	(44)
Angiectasis	1 (2%)		1 (2%)	
Depletion lymphoid			1 (2%)	
Hematopoietic cell proliferation	20 (42%)	15 (33%)	18 (39%)	14 (32%)
Hyperplasia, lymphoid	24 (50%)	21 (47%)	20 (43%)	22 (50%)
Pigmentation			1 (2%)	
Thymus	(41)	(39)	(37)	(40)
Atrophy	22 (54%)	19 (49%)	17 (46%)	23 (58%)
Hyperplasia, lymphoid	2 (5%)	1 (3%)		1 (3%)
Mineralization			1 (3%)	
Integumentary System				
Skin	(48)	(47)	(46)	(48)
Fibrosis		1 (2%)	4 (9%)	
Hemorrhage				1 (2%)
Hyperplasia, basal cell			1 (2%)	
Inflammation, suppurative	1 (2%)	2 (4%)	3 (7%)	1 (2%)
Inflammation, chronic			1 (2%)	
Inflammation, chronic active	1 (2%)	3 (6%)	4 (9%)	2 (4%)
Metaplasia, osseous			1 (2%)	
Mineralization	1 (2%)	1 (2%)	1 (2%)	
Necrosis				1 (2%)
Ulcer	1 (2%)		4 (9%)	3 (6%)
Epithelium, hyperplasia	1 (2%)	6 (13%)	4 (9%)	4 (8%)
Musculoskeletal System				
Skeletal muscle	(1)	(1)	(3)	(0)
Nervous System				
Brain, cerebrum	(47)	(46)	(46)	(47)
Mineralization	29 (62%)	24 (52%)	24 (52%)	24 (56%)
Respiratory System				
Lung	(48)	(47)	(46)	(47)
Congestion		1 (2%)		
Infiltration cellular, histiocyte	5 (10%)		2 (4%)	
Infiltration cellular, lymphocyte				1 (2%)
Inflammation, chronic active	1 (2%)			
Thromubs		1 (2%)		
Alveolar epithelium, hyperplasia		5 (11%)	4 (9%)	2 (4%)
Nose	(48)	(47)	(47)	(47)
Hyaline droplet	6 (13%)	31 (66%)	39 (83%)	13 (28%)
Posterior to upper incisor, dysplasia	1 (2%)		1 (2%)	
Trachea	(48)	(47)	(46)	(45)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Special Senses System				
Eye	(47)	(43)	(44)	(43)
Cataract			1 (2%)	1 (2%)
Cornea, hyperplasia		1 (2%)		
Cornea, inflammation, chronic active		1 (2%)		
Harderian gland	(48)	(44)	(46)	(44)
Infiltration cellular, lymphocyte	1 (2%)	2 (5%)	3 (7%)	2 (5%)
Inflammation, chronic active				1 (2%)
Acinus, degeneration		1 (2%)		1 (2%)
Acinus, dilatation				1 (2%)
Epithelium, hyperplasia			1 (2%)	
Urinary System				
Kidney	(48)	(45)	(46)	(45)
Cyst		3 (7%)	2 (4%)	
Cyst multilocular				1 (2%)
Fibrosis			1 (2%)	
Hyaline droplet			3 (7%)	
Infiltration cellular, lymphocyte	10 (21%)	8 (18%)	10 (22%)	12 (27%)
Inflammation, chronic active				1 (2%)
Metaplasia, osseous	2 (4%)		2 (4%)	
Nephropathy	26 (54%)	18 (40%)	17 (37%)	20 (44%)
Pigmentation			1 (2%)	
Pelvis, dilatation		1 (2%)	1 (2%)	
Urethra	(1)	(0)	(0)	(1)
Bulbourethral gland, dilatation	1 (100%)			
Bulbourethral gland, infiltration cellular, lymphocyte	1 (100%)			
Urinary bladder	(48)	(45)	(45)	(44)
Infiltration cellular, lymphocyte	3 (6%)	2 (4%)	2 (4%)	2 (5%)
Inflammation, chronic active				1 (2%)
Lumen, dilatation	3 (6%)	1 (2%)	1 (2%)	1 (2%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF ALOE VERA WHOLE LEAF EXTRACT

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract[a]

	0%	1%	2%	3%
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund sacrifice	6	12	8	6
Natural death	5	4	3	5
Survivors				
Moribund sacrifice	1	1		1
Natural death		1	1	2
Terminal sacrifice	35	30	36	34
Animals examined microscopically	47	48	48	48
Alimentary System				
Gallbladder	(42)	(43)	(44)	(41)
Lymphoma malignant	2 (5%)	1 (2%)	1 (2%)	
Intestine large, ascending colon	(43)	(43)	(44)	(43)
Intestine large, cecum	(42)	(43)	(44)	(42)
Lymphoma malignant	2 (5%)	1 (2%)	1 (2%)	
Intestine large, descending colon	(43)	(43)	(44)	(43)
Intestine large, rectum	(43)	(43)	(44)	(42)
Fibrosarcoma, metastatic, skin	1 (2%)			
Lymphoma malignant			1 (2%)	1 (2%)
Intestine large, transverse colon	(42)	(42)	(44)	(43)
Intestine small, duodenum	(43)	(43)	(44)	(42)
Adenoma	2 (5%)	2 (5%)	1 (2%)	1 (2%)
Lymphoma malignant	1 (2%)			
Intestine small, ileum	(42)	(43)	(44)	(42)
Lymphoma malignant	3 (7%)		1 (2%)	1 (2%)
Intestine small, jejunum	(42)	(43)	(44)	(42)
Lymphoma malignant	1 (2%)			1 (2%)
Liver	(45)	(44)	(46)	(46)
Hemangiosarcoma				1 (2%)
Hepatocellular adenoma	2 (4%)	5 (11%)	1 (2%)	4 (9%)
Hepatocellular carcinoma	3 (7%)	5 (11%)		2 (4%)
Hepatocholangiocarcinoma		1 (2%)		
Histiocytic sarcoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Leukemia			1 (2%)	2 (4%)
Lymphoma malignant	9 (20%)	8 (18%)	5 (11%)	1 (2%)
Osteosarcoma, metastatic, bone, femur			1 (2%)	1 (2%)
Mesentery	(6)	(8)	(9)	(3)
Lymphoma malignant	1 (17%)			
Osteosarcoma, metastatic, bone, femur			1 (11%)	
Oral mucosa	(1)	(0)	(0)	(0)
Squamous cell carcinoma	1 (100%)			
Pancreas	(42)	(43)	(44)	(43)
Histiocytic sarcoma		1 (2%)		
Lymphoma malignant	4 (10%)	3 (7%)	2 (5%)	2 (5%)
Salivary glands	(43)	(43)	(44)	(43)
Histiocytic sarcoma		1 (2%)		
Lymphoma malignant	7 (16%)	4 (9%)	4 (9%)	4 (9%)
Stomach, forestomach	(43)	(44)	(45)	(42)
Lymphoma malignant	1 (2%)			
Squamous cell papilloma		2 (5%)	2 (4%)	3 (7%)
Stomach, glandular	(43)	(44)	(45)	(42)
Lymphoma malignant	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Cardiovascular System				
Heart	(45)	(46)	(46)	(44)
Histiocytic sarcoma			1 (2%)	
Leukemia				1 (2%)
Lymphoma malignant	1 (2%)			
Osteosarcoma, metastatic, bone, femur			1 (2%)	
Endocrine System				
Adrenal cortex	(44)	(43)	(44)	(44)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Leukemia				1 (2%)
Lymphoma malignant	3 (7%)	2 (5%)	3 (7%)	
Adrenal medulla	(44)	(42)	(44)	(44)
Pheochromocytoma benign				1 (2%)
Islets, pancreatic	(42)	(43)	(44)	(43)
Lymphoma malignant		1 (2%)	2 (5%)	1 (2%)
Parathyroid gland	(42)	(37)	(42)	(40)
Pituitary gland	(43)	(38)	(41)	(44)
Leukemia			1 (2%)	2 (5%)
Pars distalis, adenoma	14 (33%)	10 (26%)	6 (15%)	3 (7%)
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(43)	(43)	(44)	(43)
Follicular cell, adenoma		1 (2%)		
General Body System				
None				
Genital System				
Clitoral gland	(44)	(44)	(44)	(42)
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant	1 (2%)			
Ovary	(44)	(46)	(45)	(44)
Cystadenoma			1 (2%)	
Granulosa cell tumor benign		1 (2%)	1 (2%)	1 (2%)
Histiocytic sarcoma			1 (2%)	1 (2%)
Luteoma			1 (2%)	
Lymphoma malignant	4 (9%)	4 (9%)	2 (4%)	1 (2%)
Uterus	(46)	(45)	(44)	(45)
Hemangioma		1 (2%)	1 (2%)	
Hemangiosarcoma	1 (2%)			
Histiocytic sarcoma	2 (4%)		1 (2%)	1 (2%)
Leiomyosarcoma			1 (2%)	
Lymphoma malignant	3 (7%)		3 (7%)	1 (2%)
Polyp stromal	1 (2%)	1 (2%)	5 (11%)	1 (2%)
Sarcoma	2 (4%)			
Hematopoietic System				
Bone marrow	(43)	(43)	(44)	(43)
Hemangiosarcoma				1 (2 %)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Leukemia			1 (2%)	2 (5%)
Lymphoma malignant	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Hematopoietic System (continued)				
Lymph node	(14)	(15)	(12)	(13)
Lymphoma malignant	1 (7%)			
Axillary, lymphoma malignant	5 (36%)	4 (27%)	1 (8%)	2 (15%)
Deep cervical, histiocytic sarcoma				1 (8%)
Inguinal, lymphoma malignant	1 (7%)	2 (13%)		1 (8%)
Inguinal, osteosarcomas, metastatic, bone, femur			1 (8%)	
Lumbar, histiocytic sarcoma		1 (7%)		
Lumbar, leukemia				2 (15%)
Lumbar, lymphoma malignant	10 (71%)	10 (67%)	8 (67%)	4 (31%)
Mediastinal, fibrosarcoma, metastatic, skin		1 (7%)		
Mediastinal, hepatocholangiocarcinoma, metastatic, liver		1 (7%)		
Mediastinal, histiocytic sarcoma		1 (7%)		
Mediastinal, lymphoma malignant	4 (29%)	4 (27%)	1 (8%)	2 (15%)
Pancreatic, histiocytic sarcoma		1 (7%)		
Pancreatic, lymphoma malignant	4 (29%)	1 (7%)	2 (17%)	1 (8%)
Popliteal, lymphoma malignant		1 (7%)		1 (8%)
Renal, histiocytic sarcoma		1 (7%)		
Renal, Leukemia				2 (15%)
Renal, lymphoma malignant	8 (57%)	8 (53%)	3 (25%)	4 (31%)
Lymph node, mandibular	(43)	(44)	(45)	(43)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Leukemia				1 (2%)
Lymphoma malignant	12 (28%)	10 (23%)	8 (18%)	5 (12%)
Sarcoma, metastatic, skin	1 (2%)			
Lymph node, mesenteric	(43)	(45)	(43)	(42)
Fibrous histiocytoma, metastatic, skin			1 (2%)	
Histiocytic sarcoma	1 (2%)	1 (2%)	1 (2%)	
Leukemia			1 (2%)	2 (5%)
Lymphoma malignant	16 (37%)	14 (31%)	14 (33%)	5 (12%)
Spleen	(44)	(46)	(45)	(44)
Hemangiosarcoma	3 (7%)	1 (2%)		1 (2%)
Histiocytic sarcoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Leukemia			1 (2%)	2 (5%)
Lymphoma malignant	18 (41%)	17 (37%)	17 (38%)	7 (16%)
Thymus	(41)	(44)	(43)	(41)
Lymphoma malignant	11 (27%)	8 (18%)	7 (16%)	5 (12%)
Osteosarcoma, metastatic, bone, femur			1 (2%)	
Mammary gland	(44)	(43)	(44)	(44)
Adenocarcinoma	5 (11%)	3 (7%)	2 (5%)	4 (9%)
Carcinosarcoma				1 (2%)
Lymphoma malignant	2 (5%)		1 (2%)	
Skin	(44)	(46)	(45)	(44)
Fibroma		1 (2%)		
Fibrosarcoma	2 (5%)	2 (4%)	2 (4%)	2 (5%)
Fibrous histiocytoma		1 (2%)	1 (2%)	
Hemangioma	1 (2%)			
Hemangiosarcoma				1 (2%)
Lymphoma malignant		1 (2%)	1 (2%)	
Melanoma benign	1 (2%)			1 (2%)
Sarcoma	3 (7%)			1 (2%)
Musculoskeletal System				
Bone, femur	(47)	(46)	(48)	(46)
Osteosarcoma			1 (2%)	1 (2%)
Skeletal muscle	(0)	(2)	(2)	(0)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (50%)	
Hepatocholangiocarcinoma, metastatic, liver		1 (50%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Nervous System				
Brain, brain stem	(43)	(44)	(44)	(43)
Brain, cerebellum	(43)	(44)	(44)	(43)
Brain, cerebrum	(43)	(44)	(44)	(43)
Respiratory System				
Lung	(45)	(45)	(46)	(44)
Adenocarcinoma, metastatic, mammary gland	1 (2%)			
Alveolar/bronchiolar adenoma	3 (7%)	2 (4%)	1 (2%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)		
Alveolar/bronchiolar carcinoma		3 (7%)	2 (4%)	3 (7%)
Fibrosarcoma, metastatic, skin		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Histiocytic sarcoma	2 (4%)	1 (2%)		1 (2%)
Leukemia			1 (2%)	2 (5%)
Lymphoma malignant	9 (20%)	8 (18%)	5 (11%)	3 (7%)
Osteosarcoma, metastatic, bone, femur			1 (2%)	1 (2%)
Sarcoma	1 (2%)			
Sarcoma, metastatic, skin	1 (2%)			
Nose	(45)	(44)	(45)	(45)
Special Senses System				
Eye	(42)	(43)	(44)	(42)
Harderian gland	(43)	(43)	(44)	(43)
Adenoma	3 (7%)	2 (5%)	2 (5%)	3 (7%)
Carcinoma	2 (5%)			
Lymphoma malignant			1 (2%)	
Bilateral, adenoma		1 (2%)		
Lacrimal gland	(1)	(0)	(0)	(0)
Urinary System				
Kidney	(44)	(43)	(46)	(44)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Leukemia			1 (2%)	1 (2%)
Lymphoma malignant	8 (18%)	6 (14%)	6 (13%)	1 (2%)
Osteosarcoma, metastatic, bone, femur			1 (2%)	
Urinary bladder	(43)	(43)	(44)	(42)
Histiocytic sarcoma		1 (2%)		
Lymphoma malignant	5 (12%)	3 (7%)	3 (7%)	
Systemic Lesions				
Multiple organs	(47)[b]	(48)[b]	(48)[b]	(48)[b]
Histiocytic sarcoma	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Leukemia			1 (2%)	2 (4%)
Lymphoma malignant	18 (38%)	20 (42%)	18 (38%)	8 (17%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Neoplasm Summary				
Total animals with primary neoplasms[c]	41	40	35	32
Total primary neoplasms	72	67	51	48
Total animals with benign neoplasms	23	19	18	16
Total benign neoplasms	28	30	22	19
Total animals with malignant neoplasms	37	33	28	23
Total malignant neoplasms	44	37	29	29
Total animals with metastatic neoplasms	4	2	3	1
Total metastatic neoplasms	4	5	9	2

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Statistical Analysis of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	1%	2%	3%
All Organs: Lymphoma Malignant				
Overall rate[a]	18/47 (38%)	20/48 (42%)	18/48 (38%)	8/48 (17%)
Adjusted rate[b]	41.3%	45.7%	40.1%	19.3%
Terminal rate[c]	15/35 (43%)	11/30 (37%)	15/36 (42%)	7/34 (21%)
First incidence (days)[d]	666	561	570	674
Poly-3 test[e]	P=0.020N	P=0.420	P=0.539N	P=0.021N
All Organs: Histiocytic Sarcoma				
Overall rate	3/47 (6%)	1/48 (2%)	1/48 (2%)	1/48 (2%)
Adjusted rate	6.8%	2.4%	2.3%	2.4%
Terminal rate	1/35 (3%)	0/30 (0%)	0/36 (0%)	0/34 (0%)
First incidence (days)	542	695	559	723
Poly-3 test	P=0.182N	P=0.325N	P=0.302N	P=0.327N
All Organs: Osteosarcoma				
Overall rate	0/47 (0%)	0/48 (0%)	1/48 (2%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	2.3%	2.4%
Terminal rate	0/35 (0%)	0/30 (0%)	0/36 (0%)	1/34 (3%)
First incidence (days)	----	----	630	728 (T)
Poly-3 test	P=0.173	---	P=0.505	P=0.492
All Organs: Malignant Neoplasms				
Overall rate	37/47 (79%)	33/48 (69%)	28/48 (58%)	23/48 (48%)
Adjusted rate	80.1%	71.8%	58.6%	52.1%
Terminal rate	27/35 (77%)	18/30 (60%)	17/36 (47%)	15/34 (44%)
First incidence (days)	542	535	559	547
Poly-3 test	P<.001N	P=0.242N	P=0.018N	=0.003N
All Organs: Benign Neoplasms				
Overall rate	23/47 (49%)	19/48 (40%)	18/48 (38%)	16/48 (33%)
Adjusted rate	52.1%	43.5%	40.9%	37.9%
Terminal rate	20/35 (57%)	15/30 (50%)	17/36 (47%)	13/34 (38%)
First incidence (days)	546	535	643	609
Poly-3 test	P=0.097N	P=0.275N	P=0.197N	P=0.130N
All Organs: Primary Neoplasms				
Overall rate	41/47 (87%)	40/48 (83%)	35/48 (73%)	32/48 (67%)
Adjusted rate	88.8%	84.8%	73.2%	72.4%
Terminal rate	31/35 (89%)	23/30 (77%)	24/36 (67%)	24/34 (71%)
First incidence (days)	542	535	559	547
Poly-3 test	P=0.012N	P=0.397N	P=0.045N	P=0.037N
Liver: Hepatocellular Carcinoma				
Overall rate	3/45 (7%)	5/44 (11%)	0/46 (0%)	2/46 (4%)
Adjusted rate	7.2%	12.5%	0.0%	4.9%
Terminal rate	3/35 (9%)	2/30 (7%)	0/36 (0%)	1/34 (3%)
First incidence (days)	728 (T)	535	----	673
Poly-3 test	P=0.154N	P=0.334	P=0.116N	P=0.510N
Liver: Hepatocellular Adenoma				
Overall rate	2/45 (4%)	5/44 (11%)	1/46 (2%)	4/46 (9%)
Adjusted rate	4.8%	12.5%	2.4%	9.8%
Terminal rate	2/35 (6%)	2/30 (7%)	1/36 (3%)	3/34 (9%)
First incidence (days)	728 (T)	535	728 (T)	673
Poly-3 test	P=0.447	P=0.201	P=0.497N	P=0.326

TABLE D2
Statistical Analysis of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	5/45 (11%)	9/44 (21%)	1/46 (2%)	5/46 (11%)
Adjusted rate	12.0%	21.9%	2.4%	12.3%
Terminal rate	5/35 (14%)	4/30 (13%)	1/36 (3%)	4/34 (12%)
First incidence (days)	728 (T)	535	728 (T)	673
Poly-3 test	P=0.228N	P=0.183	P=0.098N	P=0.617
Forestomach: Squamous Cell Papilloma				
Overall rate	0/43 (0%)	2/44 (5%)	2/45 (4%)	3/42 (7%)
Adjusted rate	0.0%	5.2%	4.8%	7.8%
Terminal rate	0/35 (0%)	2/30 (7%)	1/36 (3%)	2/34 (6%)
First incidence (days)	----	728 (T)	643	674
Poly-3 test	P=0.078	P=0.224	P=0.243	P=0.108
Pituitary Gland: Adenoma, Pars Distalis				
Overall rate	14/43 (33%)	10/38 (26%)	6/41 (15%)	3/44 (7%)
Adjusted rate	34.4%	30.1%	15.8%	7.7%
Terminal rate	12/34 (35%)	10/27 (37%)	6/34 (18%)	3/34 (9%)
First incidence (days)	666	728 (T)	728 (T)	728 (T)
Poly-3 test	P<.001N	P=0.444N	P=0.048N	P=0.003N
Mammary Gland: Adenocarcinoma				
Overall rate	5/44 (11%)	3/43 (7%)	2/44 (5%)	4/44 (9%)
Adjusted rate	12.1%	7.8%	4.9%	10.0%
Terminal rate	5/35 (14%)	2/30 (7%)	1/36 (3%)	1/34 (3%)
First incidence (days)	728 (T)	535	647	567
Poly-3 test	P=0.357N	P=0.391N	P=0.217N	P=0.520N
Skin: Sarcoma				
Overall rate	3/44 (7%)	0/46 (0%)	0/45 (0%)	1/44 (2%)
Adjusted rate	7.1%	0.0%	0.0%	2.6%
Terminal rate	1/35 (3%)	0/30 (0%)	0/36 (0%)	1/34 (3%)
First incidence (days)	546	----	----	728 (T)
Poly-3 test	P=0.124N	P=0.129N	P=0.121N	P=0.335N
Spleen: Hemangiosarcoma				
Overall rate	3/44 (7%)	1/46 (2%)	0/45 (0%)	1/44 (2%)
Adjusted rate	7.3%	2.5%	0.0%	2.5%
Terminal rate	3/35 (9%)	1/30 (3%)	0/36 (0%)	0/34 (0%)
First incidence (days)	728 (T)	728 (T)	----	577
Poly-3 test	P=0.110N	P=0.314N	P=0.117N	P=0.313N
Lung: Alveolar/Bronchiolar Adenoma				
Overall rate	3/45 (7%)	3/45 (7%)	1/46 (2%)	1/44 (2%)
Adjusted rate	7.2%	7.7%	2.4%	2.5%
Terminal rate	3/35 (9%)	3/30 (10%)	1/36 (3%)	1/34 (3%)
First incidence (days)	728 (T)	728 (T)	728 (T)	728 (T)
Poly-3 test	P=0.137N	P=0.632	P=0.301N	P=0.325N
Uterus: Polyp Stromal				
Overall rate	1/46 (2%)	1/45 (2%)	5/44 (11%)	1/45 (2%)
Adjusted rate	2.3%	2.6%	12.3%	2.5%
Terminal rate	0/35 (0%)	1/30 (3%)	5/36 (14%)	1/34 (3%)
First incidence (days)	546	728 (T)	728 (T)	728 (T)
Poly-3 test	P=0.291	P=0.736	P=0.087	P=0.745

TABLE D2
Statistical Analysis of Neoplasms in Female Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Harderian Gland: Adenoma				
Overall rate	3/43 (7%)	3/43 (7%)	2/44 (5%)	3/43 (7%)
Adjusted rate	7.4%	7.7%	4.9%	7.7%
Terminal rate	3/35 (9%)	0/29 (0%)	2/36 (6%)	2/34 (6%)
First incidence (days)	728 (T)	535	728 (T)	609
Poly-3 test	P=0.512N	P=0.640	P=0.500N	P=0.645

[a] Number of neoplasm-bearing animals over number of animals examined.
[b] Poly K incidence; estimated neoplasm incidence after adjustment for intercurrent mortality.
[c] Observed incidence at terminal kill.
[d] Time to first lesion in days. T indicates terminal sacrifice.
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by N.

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract[a]

	0%	1%	2%	3%
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund sacrifice	6	12	8	6
Natural death	5	4	3	5
Survivors				
Moribund sacrifice	1	1		1
Natural death		1	1	2
Terminal sacrifice	35	30	36	34
Animals examined microscopically	47	48	48	48
Alimentary System				
Gallbladder	(42)	(43)	(44)	(41)
Intestine large, ascending colon	(43)	(43)	(44)	(43)
Goblet cell, hyperplasia	1 (2%)	15 (35%)	20 (45%)	25 (58%)
Intestine large, cecum	(42)	(43)	(44)	(42)
Hyperplasia, lymphoid	1 (2%)			
Goblet cell, hyperplasia	1 (2%)		2 (5%)	2 (5%)
Intestine large, descending colon	(43)	(43)	(44)	(42)
Goblet cell, hyperplasia		4 (9%)	7 (16%)	17 (40%)
Intestine large, rectum	(43)	(43)	(44)	(42)
Intestine large, transverse colon	(42)	(42)	(44)	(43)
Goblet cell, hyperplasia	2 (5%)	18 (43%)	23 (52%)	26 (60%)
Intestine small, duodenum	(43)	(43)	(44)	(42)
Intestine small, ileum	(42)	(43)	(44)	(42)
Hyperplasia, lymphoid			1 (2%)	1 (2%)
Intestine small, jejunum	(42)	(43)	(44)	(42)
Liver	(45)	(44)	(46)	(46)
Autolysis				1 (2%)
Basophilic focus	1 (2%)		1 (2%)	4 (9%)
Clear cell focus		1 (2%)		
Cyst, multiple				1 (2%)
Cytomegaly		1 (2%)		
Eosinophilic focus	1 (2%)			1 (2%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)		2 (4%)
Infiltration cellular, lymphocyte	10 (22%)	6 (14%)	6 (13%)	9 (20%)
Inflammation, chronic active	6 (13%)	5 (11%)	4 (9%)	5 (11%)
Mixed cell focus	1 (2%)		1 (2%)	1 (2%)
Necrosis	3 (7%)	2 (5%)	2 (4%)	3 (7%)
Pigmentation	1 (2%)		1 (2%)	
Tension lipidosis	7 (16%)	5 (11%)	2 (4%)	5 (11%)
Vacuolization cytoplasmic	12 (27%)	14 (32%)	12 (26%)	16 (35%)
Bile duct, hyperplasia		1 (2%)		
Oval cell, hyperplasia	1 (2%)			
Parenchyma, degeneration			1 (2%)	
Mesentery	(6)	(8)	(9)	(3)
Angiectasis			1 (11%)	
Infiltration cellular, lymphocyte			1 (11%)	
Inflammation, chronic active			1 (11%)	
Fat, hemorrhage	1 (17%)			1 (33%)
Fat, infiltration cellular, histiocyte				1 (33%)
Fat, necrosis	6 (100%)	8 (100%)	8 (89%)	2 (67%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Alimentary System (continued)				
Oral Mucosa	(1)	(0)	(0)	(0)
Pancreas	(42)	(43)	(44)	(43)
Cyst				1 (2%)
Hemorrhage				1 (2%)
Infiltration cellular, lymphocyte	11 (26%)	4 (9%)	13 (30%)	15 (35%)
Vacuolization cytoplasmic	1 (2%)	2 (5%)		
Acinus, degeneration	1 (2%)	1 (2%)	1 (2%)	
Duct, dilatation			1 (2%)	1 (2%)
Salivary glands	(43)	(43)	(44)	(43)
Infiltration cellular, lymphocyte	29 (67%)	31 (72 %)	31 (70%)	26 (60%)
Stomach, forestomach	(43)	(44)	(45)	(42)
Keratin cyst	1 (2%)	1 (2%)		
Epithelium, hyperplasia		2 (5%)	1 (2%)	1 (2%)
Stomach, glandular	(43)	(44)	(45)	(42)
Cyst	1 (2%)			
Erosion				1 (2%)
Inflammation, chronic active		1 (2%)		
Epithelium, hyperplasia		1 (2%)	3 (7%)	4 (10%)
Cardiovascular System				
Heart	(45)	(46)	(46)	(44)
Cardiomyopathy	4 (9%)	2 (4%)	3 (7%)	
Inflammation, chronic active			1 (2%)	
Mineralization	1 (2%)			
Endocrine System				
Adrenal cortex	(44)	(43)	(44)	(44)
Vacuolization cytoplasmic	4 (9%)	4 (9%)	1 (2%)	1 (2%)
Subcapsular, hyperplasia	43 (98%)	43 (100%)	43 (98%)	44 (100)
Adrenal medulla	(44)	(42)	(44)	(44)
Hyperplasia	1 (2%)		1 (2%)	
Pigmentation				1 (2%)
Islets, pancreatic	(42)	(43)	(44)	(43)
Hyperplasia	1 (2%)	5 (12%)	1 (2%)	2 (5%)
Parathyroid gland	(42)	(37)	(42)	(40)
Cyst		1 (3%)	1 (2%)	
Infiltration cellular, lymphocyte				1 (3%)
Pituitary gland	(43)	(38)	(41)	(44)
Thrombus			1 (2%)	
Pars distalis, angiectasis			1 (2%)	1 (2%)
Pars distalis, cyst			1 (2%)	2 (5%)
Pars distalis, hyperplasia	7 (16%)	8 (21%)	10 (24%)	11 (25%)
Pars intermedia, angiectasis			1 (2%)	
Thyroid gland	(43)	(43)	(44)	(43)
Cyst		1 (2%)	1 (2%)	
Ectopic thymus	2 (5%)	1 (2%)		
Infiltration cellular, lymphocyte	3 (7%)	1 (2%)	2 (5%)	6 (14%)
Inflammation, suppurative				1 (2%)
Inflammation, chronic active			1 (2%)	
Follicle, degeneration	6 (14%)	10 (23%)	9 (20%)	6 (14%)
Follicular cell, hyperplasia	3 (7%)	1 (2%)	1 (2%)	
Follicular cell, hypertrophy	1 (2%)			

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
General Body System				
None				
Genital System				
Clitoral Gland	(44)	(44)	(44)	(42)
Degeneration	43 (98%)	44 (100%)	43 (98%)	40 (95%)
Ovary	(44)	(46)	(45)	(44)
Atrophy	40 (91%)	39 (85%)	40 (89%)	39 (89%)
Autolysis		1 (2%)		
Cyst	14 (32%)	18 (39%)	12 (27%)	9 (20%)
Cyst, multiple	1 (2%)		2 (4%)	2 (5%)
Hemorrhage			1 (2%)	
Uterus	(46)	(45)	(44)	(45)
Angiectasis			1 (2%)	
Autolysis	1 (2%)			1 (2%)
Edema	1 (2%)			
Hydrometra		1 (2%)		1 (2%)
Thrombus		1 (2%)	1 (2%)	1 (2%)
Endometrium, hyperplasia, cystic	43 (93%)	44 (98%)	43 (98%)	43 (96%)
Lumen, dilatation	1 (2%)			
Hematopoietic System				
Bone marrow	(43)	(43)	(44)	(43)
Fibrosis	1 (2%)			
Hyperplasia	7 (16%)	5 (12%)	5 (11%)	3 (7%)
Lymph node	(14)	(15)	(12)	(13)
Axillary, infiltration cellular, polymorphonuclear		1 (7%)		
Iliac, hyperplasia, lymphoid				1 (8%)
Iliac, infiltration cellular, plasma cell				1 (8%)
Iliac, infiltration cellular, polymorphonuclear				1 (8%)
Lumbar, hemorrhage				2 (15%)
Lumbar, hyperplasia, lymphoid	3 (21%)		1 (8%)	5 (38%)
Lumbar, infiltration cellular, plasma cell	1 (7%)		1 (8%)	
Lumbar, infiltration cellular, polymorphonuclear	2 (14%)	1 (7%)		
Lumbar, sinus, dilatation				1 (8%)
Renal, hemorrhage				1 (8%)
Renal, hyperplasia, lymphoid	2 (14%)			1 (8%)
Renal, infiltration cellular, polymorphonuclear	2 (14%)			
Lymph node, mandibular	(43)	(44)	(45)	(43)
Amyloid deposition		1 (2%)		
Hyperplasia, lymphoid	11 (26%)	13 (20%)	12 (27%)	14 (33%)
Infiltration cellular, lymphocyte		1 (2%)		
Infiltration cellular, plasma cell	1 (2%)	2 (5%)		
Infiltration cellular, polymorphonuclear	1 (2%)		1 (2%)	
Lymph node, mesenteric	(43)	(45)	(43)	(42)
Amyloid deposition		1 (2%)		
Angiectasis	1 (2%)		1 (2%)	
Hemorrhage		1 (2%)		2 (5%)
Hyperplasia, lymphoid	10 (23%)	12 (27%)	14 (33%)	16 (38%)
Infiltration cellular, plasma cell	1 (2%)	2 (4%)		
Sinus, dilatation				1 (2%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Hematopoietic System (continued)				
Spleen	(44)	(46)	(45)	(44)
Amyloid deposition		1 (2%)		
Depletion lymphoid				1 (2%)
Fibrosis, stromal	1 (2%)			
Hematopoietic cell proliferation	16 (36%)	13 (28%)	9 (20%)	12 (27%)
Hyperplasia, lymphoid	20 (45%)	23 (50%)	21 (47%)	24 (55%)
Necrosis			1 (2%)	
Pigmentation	3 (7%)	4 (9%)	4 (9%)	5 (11%)
Thrombus	1 (2%)			
Thymus	(41)	(44)	(43)	(41)
Amyloid deposition		1 (2%)		
Atrophy	10 (24%)	14 (32%)	17 (40%)	10 (24%)
Cyst				1 (2%)
Hyperplasia, lymphoid	8 (20%)	7 (16%)	8 (19%)	12 (29%)
Necrosis	1 (2%)			
Epithelial cell, hyperplasia				1 (2%)
Integumentary System				
Mammary gland	(44)	(43)	(44)	(44)
Galactocele	1 (2%)		1 (2%)	
Infiltration cellular, lymphocyte			1 (2%)	1 (2%)
Lactation	2 (5%)		2 (5%)	
Alveolus, hyperplasia	4 (9%)	3 (7%)	3 (7%)	3 (7%)
Skin	(44)	(46)	(45)	(44)
Fat, necrosis			1 (2%)	
Musculoskeletal System				
Bone, femur	(47)	(46)	(48)	(46)
Fibro-osseous lesion	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Skeletal muscle	(0)	(2)	(2)	(0)
Diaphragm, inflammation, chronic active			1 (50%)	
Nervous System				
Brain, brain stem	(43)	(44)	(44)	(43)
Compression	2 (5%)	1 (2%)	2 (5%)	1 (2%)
Brain, cerebellum	(43)	(44)	(44)	(43)
Infiltration cellular, lymphocyte				1 (2%)
Brain, cerebrum	(43)	(44)	(44)	(43)
Infiltration cellular, lymphocyte				1 (2%)
Mineralization	24 (56%)	30 (68%)	24 (55%)	27 (63%)
Respiratory System				
Lung	(45)	(45)	(46)	(44)
Autolysis	1 (2%)		1 (2%)	
Congestion				1 (2%)
Hemorrhage	2 (4%)	1 (2%)		
Infiltration cellular, histiocyte	5 (11%)	4 (9%)	3 (7%)	1 (2%)
Infiltration cellular, lymphocyte	4 (9%)	2 (4%)	2 (4%)	3 (7%)
Inflammation, chronic		1 (2%)		
Inflammation, chronic active	2 (4%)	1 (2%)		1 (2%)
Metaplasia, osseous		1 (2%)		
Mineralization			1 (2%)	
Alveolar epithelium, hyperplasia			2 (4%)	1 (2%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Respiratory System (continued)				
Nose	(45)	(44)	(45)	(45)
Hyaline droplet	12 (27%)	12 (27%)	17 (38%)	9 (20%)
Inflammation, chronic active	1 (2%)			
Special Senses System				
Eye	(42)	(43)	(44)	(42)
Cataract	2 (5%)		2 (5%)	
Phthisis bulbi		1 (2%)		
Cornea, inflammation, chronic active	1 (2%)			
Cornea, ulcer	1 (2%)			
Harderian gland	(43)	(43)	(44)	(43)
Hyperlasia				1 (2%)
Infiltration cellular, lymphocyte	6 (14%)	5 (12%)	5 (11%)	5 (12%)
Epithelium, hyperplasia		2 (5%)		
Lacrimal gland	(1)	(0)	(0)	(0)
Infiltration cellular, lymphocyte	1 (100%)			
Urinary System				
Kidney	(44)	(43)	(46)	(44)
Amyloid deposition	1 (2%)	1 (2%)		2 (5%)
Autolysis	1 (2%)			1 (2%)
Cyst, multiple		1 (2%)		
Hyaline droplet	3 (7%)	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, histiocyte	1 (2%)			
Infiltration cellular, lymphocyte	22 (50%)	23 (53%)	22 (48%)	27 (61%)
Metaplasia, osseous			3 (7%)	2 (5%)
Nephropathy	5 (11%)	6 (14%)	7 (15%)	6 (14%)
Polyarteritis				1 (2%)
Pelvis, dilatation		1 (2%)	2 (4%)	
Pelvis, mineralization		1 (2%)		
Transitional epithelium, hyperplasia		1 (2%)		
Urinary bladder	(43)	(43)	(44)	(42)
Infiltration cellular, lymphocyte	22 (51%)	22 (51%)	22 (50%)	25 (60%)
Lumen, dilatation			1 (2%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

APPENDIX E
CLINICAL PATHOLOGY RESULTS

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 14-Day Drinking Water Study of Aloe vera Extracts[a]

	0%	0.5%	1.0%	1.5%	2.0%	3.0%
Gel Extract						
Male						
Leukocyte Cell Count ($10^3/\mu l$)[b]	4.9 ± 0.7	5.5 ± 0.73	7.0 ± 1.0	6.4 ± 0.7	5.3 ± 1.0	6.0 ± 0.7
Erythrocyte Cell Count ($10^3/\mu l$)[b]	7.93 ± 0.14	7.86 ± 0.14	8.41 ± 0.20	8.07 ± 0.14	8.37 ± 0.20	7.83 ± 0.14
Hemoglobin (g/dl)[b]	16.0 ± 0.3	15.7 ± 0.3	15.2 ± 0.5	16.1 ± 0.3	15.4 ± 0.5	15.8 ± 0.3
Hematocrit (%)[b]	44.1 ± 0.8	43.9 ± 0.8	44.5 ± 1.1	43.7 ± 0.8	45.7 ± 1.1	42.5 ± 0.8
Mean Cell Volume (μm^3)[b]	56 ± 0	55 ± 0	55 ± 0	55 ± 0	55 ± 0	55 ± 0
Mean Cell Hemoglobin (pg)[b]	20.2 ± 0.5	20.1 ± 0.5	18.70 ± 0.76	19.93 ± 0.53	18.60 ± 0.76	20.18 ± 0.53
Mean Cell Hemoglobin Concentration (g/dl)[b]	36.4 ± 0.9	36.4 ± 0.9	34.15 ± 1.30	36.48 ± 0.92	33.85 ± 1.30	36.25 ± 0.92
Platelets ($10^3/\mu l$)[b]	699 ± 25	765 ± 25	815 ± 36	732 ± 25	756 ± 36	690 ± 25
Cholesterol (mg/dl)[c]	87 ± 6*	78 ± 6	82 ± 6	74 ± 6	72 ± 6	65 ± 6
Triglycerides (mg/dl)[c]	92 ± 7*	74 ± 7	85 ± 7	79 ± 7	67 ± 7	59 ± 7*
Blood Urea Nitrogen (mg/dl)[c]	22.8 ± 7.0	14.1 ± 7.0	14.3 ± 7.0	26.0 ± 7.0	23.2 ± 7.0	17.1 ± 7.0
Creatinine (mg/dl)[c]	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.1
Glucose (mg/dl)[c]	100 ± 5	99 ± 5	85 ± 5	94 ± 5	104 ± 5	101 ± 5
Total Protein (g/dl)[c]	6.4 ± 0.2*	6.0 ± 0.2	6.1 ± 0.2	6.0 ± 0.2	6.0 ± 0.2	5.7 ± 0.2*
Albumin (g/dl)[c]	4.4 ± 0.1*	4.2 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.0 ± 0.1
Alanine Aminotransferase (U/l)[e]	55 ± 3	53 ± 3	56 ± 3	51 ± 3	52 ± 3	49 ± 3
Aspartate Aminotransferase (U/l)[c]	96 ± 8	84 ± 8	100 ± 8	105 ± 8	95 ± 8	76 ± 8
Amylase (U/l)[c]	1537 ± 65	1358 ± 65	1464 ± 65	1421 ± 65	1509 ± 65	1421 ± 65
Creatine Kinase (U/l)[c]	243 ± 43*	204 ± 43	259 ± 43	201 ± 43	133 ± 43	159 ± 43
Calcium (mg/dl)[c]	11.2 ± 0.4	10.7 ± 0.4	11.2 ± 0.4	11.5 ± 0.4	12.1 ± 0.4	10.8 ± 0.4
Inorganic Phosphorus (mg/dl)[c]	10.9 ± 0.7	9.6 ± 0.7	10.5 ± 0.7	11.0 ± 0.7	11 ± 0.7	11.0 ± 0.7
Sodium (mmol/l)[c]	150 ± 1*	149 ± 1	151 ± 1	150 ± 1	150 ± 1	148 ± 1
Potassium (mmol/l)[c]	6.1 ± 0.1	6.0 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.1 ± 0.1
Chloride (mmol/l)[c]	97 ± 1	97 ± 1	99 ± 1	99 ± 1	98 ± 1	98 ± 1
Female						
Leukocyte Cell Count ($10^3/\mu l$)[d]	4.8 ± 0.7	4.37 ± 0.77	5.43 ± 0.83	5.08 ± 0.72	4.91 ± 0.72	5.80 ± 0.77
Erythrocyte Cell Count ($10^3/\mu l$)[d]	7.70 ± 0.15	7.91 ± 0.16	7.98 ± 0.17	7.85 ± 0.15	7.92 ± 0.15	7.90 ± 0.16
Hemoglobin (g/dl)[d]	15.2 ± 0.3	16.1 ± 0.4	15.8 ± 0.4	16.0 ± 0.3	15.8 ± 0.3	15.8 ± 0.4
Hematocrit (%)[d]	42.8 ± 0.7	43.4 ± 0.8	43.6 ± 0.8	43.2 ± 0.7	43.7 ± 0.7	42.9 ± 0.8
Mean Cell Volume (μm^3)[d]	55 ± 0	56 ± 1	55 ± 1	55 ± 0	56 ± 0	55 ± 1
Mean Cell Hemoglobin (pg)[d]	19.8 ± 0.5	20.7 ± 0.6	20.0 ± 0.6	20.3 ± 0.5	20.3 ± 0.5	20.3 ± 0.6
Mean Cell Hemoglobin Concentration (g/dl)[d]	35.5 ± 0.9	37.1 ± 1.0	36.3 ± 1.0	36.8 ± 0.9	36.5 ± 0.9	36.4 ± 1.0
Platelets ($10^3/\mu l$)[d]	715 ± 35	684 ± 38	756 ± 41	745 ± 35	737 ± 35	747 ± 38
Cholesterol (mg/dl)[c]	120 ± 8*	101 ± 8	111 ± 8	104 ± 8	97 ± 8	94 ± 8
Triglycerides (mg/dl)[c]	99 ± 7*	80 ± 7	81 ± 7	63 ± 7*	61 ± 7*	65 ± 7*
Blood Urea Nitrogen (mg/dl)[c]	22.7 ± 6.8	25.0 ± 6.8	15.3 ± 6.8	19.4 ± 6.8	13.6 ± 6.8	31.2 ± 6.8
Creatinine (mg/dl)[c]	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

	0%	0.5%	1.0%	1.5%	2.0%	3.0%
Female (continued)						
Glucose (mg/dl)[e]	97 ± 7	94 ± 7	107 ± 7	106 ± 7	109 ± 7	107 ± 7
Total Protein (g/dl)[c]	6.2 ± 0.1*	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	5.9 ± 0.1
Albumin (g/dl)[c]	4.4 ± 0.1*	4.4 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	4.1 ± 0.1
Alanine Aminotransferase (U/l)[e]	45 ± 4	43 ± 4	45 ± 4	45 ± 4	43 ± 4	42 ± 4
Aspartate Aminotransferase (U/l)[c]	103 ± 9	86 ± 9	110 ± 9	92 ± 9	85 ± 9	87 ± 9
Amylase (U/l)[c]	989 ± 44	813 ± 44*	907 ± 44	955 ± 44	927 ± 44	944 ± 44
Creatine Kinase (U/l)[e]	280 ± 66	175 ± 66	372 ± 71	171 ± 66	165 ± 66	225 ± 66
Calcium (mg/dl)[c]	11.8 ± 0.4	11.1 ± 0.4	10.5 ± 0.4	10.4 ± 0.4	11.1 ± 0.4	11.1 ± 0.4
Inorganic Phosphorus (mg/dl)[c]	9.8 ± 0.5	9.8 ± 0.5	9.4 ± 0.5	9.8 ± 0.5	9.6 ± 0.5	9.5 ± 0.5
Sodium (mmol/l)[c]	150 ± 1	150 ± 1	150 ± 1	151 ± 0.1	150 ± 1	149 ± 1
Potassium (mmol/l)[c]	5.7 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.9 ± 0.1	5.7 ± 0.1	5.8 ± 0.1
Chloride (mmol/l)[c]	100 ± 1	99 ± 1	101 ± 1	99 ± 1	100 ± 1	100 ± 1
Decolorized Whole Leaf Extract						
Male						
Leukocyte Cell Count ($10^3/\mu l$)[f]	5.6 ± 0.8	6.0 ± 0.8	5.1 ± 0.8	6.3 ± 0.8	6.8 ± 0.6	6.6 ± 0.6
Erythrocyte Cell Count ($10^3/\mu l$)[f]	8.20 ± 0.15	8.31 ± 0.15	8.31 ± 0.15	8.44 ± 0.15	8.12 ± 0.11	8.25 ± 0.11
Hemoglobin (g/dl)[f]	15.0 ± 0.4*	15.2 ± 0.4	15.3 ± 0.4	15.4 ± 0.4	16.5 ± 0.3*	16.1 ± 0.3
Hematocrit (%)[f]	45.3 ± 0.8	46.0 ± 0.8	45.8 ± 0.8	46.5 ± 0.8	45 ± 0.6	45.4 ± 0.6
Mean Cell Volume (μm^3)[f]	55 ± 0	56 ± 0	55 ± 0	55 ± 0	55 ± 0	55 ± 0
Mean Cell Hemoglobin (pg)[f]	18.3 ± 0.6*	18.2 ± 0.6	18.4 ± 0.6	18.3 ± 0.6	20.3 ± 0.4*	19.5 ± 0.4
Mean Cell Hemoglobin Concentration (g/dl)[f]	33.2 ± 1.1*	32.9 ± 1.1	33.3 ± 1.1	33.2 ± 1.1	36.7 ± 0.9	35.6 ± 0.9
Platelets ($10^3/\mu l$)[f]	651 ± 66	689 ± 66	703 ± 66	671 ± 66	575 ± 50	682 ± 50
Cholesterol (mg/dl)[e]	84 ± 6	76 ± 6	83 ± 6	77 ± 6	80 ± 6	78 ± 6
Triglycerides (mg/dl)[c]	91 ± 10*	92 ± 10	100 ± 10	92 ± 10	82 ± 10	68 ± 10
Blood Urea Nitrogen (mg/dl)[c]	14.7 ± 0.5*	15.6 ± 0.5	15.0 ± 0.5	15.0 ± 0.5	13.7 ± 0.5	14.1 ± 0.5
Creatinine (mg/dl)[c]	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Glucose (mg/dl)[c]	108 ± 6	93.38 ± 5.95	104.50 ± 5.95	101.63 ± 5.95	113.63 ± 5.95	98.13 ± 5.95
Total Protein (g/dl)[c]	6.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.2 ± 0.1
Albumin (g/dl)[c]	4.5 ± 0.1*	4.5 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.3 ± 0.1	4.3 ± 0.1
Alanine Aminotransferase (U/l)[c]	46 ± 4	57 ± 4	55 ± 4	59 ± 4	54 ± 4	49 ± 4
Aspartate Aminotransferase (U/l)[c]	75 ± 10	84 ± 10	80 ± 10	85 ± 10	111 ± 10	82 ± 10
Amylase (U/l)[c]	1480 ± 61	1475 ± 61	1589 ± 61	1498 ± 61	1433 ± 61	1404 ± 61
Creatine Kinase (U/l)[c]	126 ± 68*	167 ± 68	148 ± 68	139 ± 68	438 ± 68	213 ± 68
Calcium (mg/dl)[c]	11.1 ± 0.4	10.8 ± 0.4	11.7 ± 0.4	11.5 ± 0.4	11.1 ± 0.4	11.5 ± 0.4
Inorganic Phosphorus (mg/dl)[c]	10.0 ± 0.3	10.3 ± 0.3	10.6 ± 0.3	10.2 ± 0.3	10.3 ± 0.3	10.6 ± 0.3
Sodium (mmol/l)[c]	149 ± 1*	150 ± 1	149 ± 1	149 ± 1	149 ± 1	148 ± 1
Potassium (mmol/l)[c]	6.2 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.3 ± 01	6.2 ± 0.1
Chloride (mmol/l)[c]	98 ± 1	97 ± 1	97 ± 1	98 ± 1	98 ± 1	97 ± 1

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

	0%	0.5%	1.0%	1.5%	2.0%	3.0%
Female						
Leukocyte Cell Count ($10^3/\mu l$)[g]	4.4 ± 0.9	5.6 ± 0.8	7.4 ± 0.8	5.0 ± 0.9	6.8 ± 0.8	5.5 ± 0.8
Erythrocyte Cell Count ($10^3/\mu l$)[g]	8.21 ± 0.31	8.04 ± 0.29	7.95 ± 0.29	8.38 ± 0.31	8.39 ± 0.29	7.35 ± 0.29
Hemoglobin (g/dl)[g]	16.8 ± 0.7	16.3 ± 0.7	15.9 ± 0.7	17.0 ± 0.7	17.1 ± 0.7	14.7 ± 0.7
Hematocrit (%)[g]	45.8 ± 1.7	44.6 ± 1.6	43.7 ± 1.6	46.1 ± 1.7	46.2 ± 1.6	40.8 ± 1.6
Mean Cell Volume (μm^3)[g]	56 ± 0	55 ± 0	55 ± 0	55 ± 0	55 ± 0	56 ± 0
Mean Cell Hemoglobin (pg)[g]	20.5 ± 0.6	20.2 ± 0.6	20.1 ± 0.6	20.3 ± 0.6	20.4 ± 0.6	20.1 ± 0.6
Mean Cell Hemoglobin Concentration (g/dl)[g]	36.7 ± 1.1	36.5 ± 1.0	36.5 ± 1.0	36.9 ± 1.1	37.1 ± 1.0	36.1 ± 1.0
Platelets ($10^3/\mu l$)[g]	691 ± 50	701 ± 46	588 ± 46	700 ± 50	682 ± 46	585 ± 46
Cholesterol (mg/dl)[c]	105 ± 8	110 ± 8	103 ± 8	98 ± 8	108 ± 8	103 ± 8
Triglycerides (mg/dl)[c]	86 ± 9*	70 ± 9	74 ± 9	81 ± 9	72 ± 9	59 ± 9
Blood Urea Nitrogen (mg/dl)[c]	17.5 ± 0.7*	15.6 ± 0.7	16.0 ± 0.7	14.7 ± 0.7*	15 ± 0.7*	14.2 ± 0.7*
Creatinine (mg/dl)[c]	0.7 ± 0.0*	0.7 ± 0.0	0.6 ± 0.0*	0.7 ± 0.0	0.7 ± 0.0	0.5 ± 0.0*
Glucose (mg/dl)[c]	98 ± 7	115 ± 7	109 ± 7	101 ± 7	110 ± 7	100 ± 7
Total Protein (g/dl)[c]	6.3 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.2 ± 0.1
Albumin (g/dl)[c]	4.6 ± 0.1*	4.6 ± 0.1	6.0 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.4 ± 0.1
Alanine Aminotransferase (U/l)[c]	47 ± 2*	46 ± 2	50 ± 2	46 ± 2	46 ± 2	40 ± 2
Aspartate Aminotransferase (U/l)[c]	95 ± 7	90 ± 7	93 ± 7	90 ± 7	97 ± 7	88 ± 7
Amylase (U/l)[c]	883 ± 48	1033 ± 48	992 ± 48	980 ± 48	1004 ± 48	928 ± 48
Creatine Kinase (U/l)[c]	294 ± 41	179 ± 41	191 ± 41	233 ± 41	230 ± 41	201 ± 41
Calcium (mg/dl)[c]	11.3 ± 0.6	12.3 ± 0.6	11.5 ± 0.6	11.6 ± 0.6	11.9 ± 0.6	12.0 ± 0.6
Inorganic Phosphorus (mg/dl)[c]	10.8 ± 0.3*	10.3 ± 0.3	10.4 ± 0.3	9.8 ± 0.3	10.3 ± 0.3	9.9 ± 0.3
Sodium (mmol/l)[c]	151 ± 1	150 ± 1	149 ± 1	150 ± 1	150 ± 1	149 ± 1
Potassium (mmol/l)[c]	6.15 ± 0.09*	5.91 ± 0.09	6.20 ± 0.09	5.96 ± 0.09	6.00 ± 0.09	5.9 ± 0.1
Chloride (mmol/l)[c]	98 ± 0	99 ± 0	99 ± 0	98 ± 0	99 ± 0.	98 ± 0
Whole Leaf Extract						
Male						
Leukocyte Cell Count ($10^3/\mu l$)[h]	6.5 ± 0.8*	5.7 ± 0.8	5.8 ± 0.8	8.9 ± 1.2	13.7 ± 1.2*	13.7 ± 1.2*
Erythrocyte Cell Count ($10^3/\mu l$)[h]	7.99 ± 0.10*	8.02 ± 0.10	7.78 ± 0.10	8.10 ± 0.14	8.18 ± 0.14	8.54 ± 0.14*
Hemoglobin (g/dl)[h]	16.3 ± 0.4	16.2 ± 0.4	15.8 ± 0.4	15.0 ± 0.6	15.5 ± 0.6	15.9 ± 0.6
Hematocrit (%)[h]	44.4 ± 0.5*	44.8 ± 0.5	43.1 ± 0.5	44.9 ± 0.7	45.2 ± 0.7	46.9 ± 0.7*
Mean Cell Volume (μm^3)[h]	56 ± 0*	56 ± 0	55 ± 0	56 ± 0	56 ± 0	55 ± 0
Mean Cell Hemoglobin (pg)[h]	20.4 ± 0.5*	20.2 ± 0.5	20.3 ± 0.5	18.5 ± 0.8	18.9 ± 0.8	18.7 ± 0.8
Mean Cell Hemoglobin Concentration (g/dl)[h]	36.7 ± 0.9*	36.2 ± 0.9	36.6 ± 0.9	33.4 ± 1.3	34.2 ± 1.3	34.0 ± 1.3
Platelets ($10^3/\mu l$)[h]	752 ± 53	790 ± 53	699 ± 53	859 ± 74	824 ± 74	722 ± 74
Cholesterol (mg/dl)[c]	79 ± 6	78 ± 6	72 ± 6	76 ± 6	83 ± 6	89 ± 6

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

	0%	0.5%	1.0%	1.5%	2.0%	3.0%
Male (continued)						
Triglycerides (mg/dl)[c]	94 ± 9	82 ± 8	72 ± 8	66 ± 8	81 ± 8	80 ± 8
Blood Urea Nitrogen (mg/dl)[c]	14.3 ± 1.0*	13.09 ± 1.00	13.64 ± 1.00	14.90 ± 1.00	16.93 ± 1.00	18.54 ± 1.00*
Creatinine (mg/dl)[c]	0.9 ± 0.1*	0.7 ± 0.1	0.6 ± 0.1*	0.6 ± 0.1*	0.6 ± 0.1*	0.6 ± 0.1*
Glucose (mg/dl)[c]	85 ± 5	88 ± 5	84 ± 5	87 ± 5	79 ± 5	95 ± 5
Total Protein (g/dl)[c]	6.4 ± 0.1	6.1 ± 0.1	5.9 ± 0.1*	5.9 ± 0.1*	6.1 ± 0.1	6.3 ± 0.1
Albumin (g/dl)[c]	4.6 ± 0.1*	4.4 ± 0.1	4.0 ± 0.1*	4.0 ± 0.1*	4.2 ± 0.1	4.2 ± 0.1
Alanine Aminotransferase (U/l)[c]	58 ± 3	58 ± 3	55 ± 3	50 ± 3	60 ± 3	58 ± 3
Aspartate Aminotransferase (U/l)[c]	110 ± 9*	103 ± 9	98 ± 9	93 ± 9	104 ± 9	87 ± 9
Amylase (U/l)[c]	1477 ± 65*	1372 ± 65	1350 ± 65	1316 ± 65	1307 ± 65	1298 ± 65
Creatine Kinase (U/l)[c]	430 ± 61*	322 ± 61	306 ± 61	229 ± 61	245 ± 61	142 ± 61*
Calcium (mg/dl)[c]	11.1 ± 0.4	11 ± 0.4	11.3 ± 0.4	11.4 ± 0.4	11.5 ± 0.4	11.0 ± 0.4
Inorganic Phosphorus (mg/dl)[c]	11.2 ± 0.8*	11.2 ± 0.8	10.5 ± 0.78	10.7 ± 0.8	10.9 ± 0.8	9.0 ± 0.8
Sodium (mmol/l)[c]	150.88 ± 0.64	149.50 ± 0.64	149.13 ± 0.64	148.25 ± 0.64*	149.13 ± 0.64	151.00 ± 0.64
Potassium (mmol/l)[c]	6.05 ± 0.11	6.28 ± 0.11	6.16 ± 0.11	6.24 ± 0.11	6.26 ± 0.11	6.20 ± 0.11
Chloride (mmol/l)[c]	96.13 ± 2.41	97.38 ± 2.41	91.13 ± 2.41	98.63 ± 2.41	98.50 ± 2.41	99.38 ± 2.41
Female						
Leukocyte Cell Count ($10^3/\mu l$)[e]	4.5 ± 0.9*	6.0 ± 0.9	5.8 ± 0.9	8.6 ± 0.9*	10.2 ± 0.9*	9.3 ± 0.9*
Erythrocyte Cell Count ($10^3/\mu l$)[e]	7.90 ± 0.17*	7.94 ± 0.17	8.02 ± 0.17	8.18 ± 0.19	8.20 ± 0.17	8.73 ± 0.17*
Hemoglobin (g/dl)[g]	16.1 ± 0.5*	16.3 ± 0.6	16.6 ± 0.5	16.5 ± 0.6	16.4 ± 0.5	18.0 ± 0.5
Hematocrit (%)[e]	42.9 ± 0.9*	43.6 ± 0.9	44.3 ± 0.9	45.0 ± 1.0	44.8 ± 0.9	48.2 ± 0.9*
Mean Cell Volume (μm^3)[e]	54 ± 0	55 ± 0	55 ± 0	55 ± 0	56 ± 0	55 ± 0
Mean Cell Hemoglobin (pg)[e]	20.4 ± 0.5	20.3 ± 0.5	20.7 ± 0.5	20.2 ± 0.6	20.1 ± 0.5	20.5 ± 0.5
Mean Cell Hemoglobin Concentration (g/dl)[e]	37.6 ± 0.9	37.0 ± 0.9	37.4 ± 0.9	36.7 ± 1.0	36.8 ± 0.9	37.2 ± 0.9
Platelets ($10^3/\mu l$)[e]	698 ± 51*	803 ± 51	771 ± 51	747 ± 54	695 ± 56	584 ± 51
Cholesterol (mg/dl)[c]	100 ± 9	97 ± 9	91 ± 9	87 ± 9	82 ± 9	87 ± 9
Triglycerides (mg/dl)[c]	73 ± 7	78 ± 7	72 ± 7	69 ± 7	71 ± 7	59 ± 7
Blood Urea Nitrogen (mg/dl)[c]	16.1 ± 1.9*	18.7 ± 1.9	17.5 ± 1.9	17.5 ± 1.9	19.9 ± 1.9	21.5 ± 1.9
Creatinine (mg/dl)[c]	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Glucose (mg/dl)[c]	96 ± 6	91 ± 6	82 ± 6	93 ± 6	102 ± 6	90 ± 6
Total Protein (g/dl)[c]	6.1 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.1 ± 0.1
Albumin (g/dl)[c]	4.4 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	3.9 ± 0.1*	3.9 ± 0.1*	4.3 ± 0.1
Alanine Aminotransferase (U/l)[c]	48 ± 4*	46 ± 4	46 ± 4	48 ± 4	57 ± 4	59 ± 4
Aspartate Aminotransferase (U/l)[c]	107 ± 11	96 ± 11	91 ± 11	113 ± 11	97 ± 11	109 ± 11
Amylase (U/l)[c]	928 ± 46	901 ± 46	839 ± 46	880 ± 46	922 ± 46	868 ± 46
Creatine Kinase (U/l)[c]	343 ± 64*	330 ± 64	238 ± 64	112 ± 64	167 ± 64	194 ± 64
Calcium (mg/dl)[c]	11.1 ± 0.5	11.1 ± 0.5	11.5 ± 0.5	11.3 ± 0.5	10.7 ± 0.5	10.4 ± 0.5
Inorganic Phosphorus (mg/dl)[c]	9.7 ± 0.6	10.6 ± 0.6	10.5 ± 0.6	9.4 ± 0.6	9.3 ± 0.6	9.5 ± 0.6
Sodium (mmol/l)[c]	150 ± 1	150 ± 1	150 ± 1	149 ± 1	149 ± 1	152 ± 1
Potassium (mmol/l)[c]	5.9 ± 0.1*	6.1 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	6.1 ± 0.1	5.7 ± 0.1
Chloride (mmol/l)[c]	99 ± 1*	98 ± 1	97 ± 1	98 ± 1	101 ± 1	101± 1

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

[a] Values are given as LS means ± standard error of the mean. An asterisk (*) denotes significance at $P \leq 0.05$; when listed under the 0% group, "*" represents the test for linear trend and when listed for the dosed groups, "*" represents comparison to the control group based on Dunnett's test.
[b] n=40
[c] n=48
[d] n=44
[e] n=47
[f] n=30
[g] n=46
[h] n=36

TABLE E2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract[a,b]

	0%	2%
Male		
Leukocyte Cell Count ($10^3/\mu l$)	7.9 ± 0.9	15.6 ± 0.9*
Neutrophils (%)	6.8 ± 3.4	32.8 ± 3.4*
Lymphocytes (%)	92.8 ± 3.6	65.2 ± 3.6*
Monocytes (%)	0.3 ± 0.2	1.3 ± 0.2*
Eosinophils (%)	0.3 ± 0.2	0.7 ± 0.2
Basophils (%)	0.0 ± 0.0	0.0 ± 0.0
Erythrocyte Cell Count ($10^3/\mu l$)	9.39 ± 0.22	10.12 ± 0.22*
Hemoglobin (g/dl)	16.2 ± 0.3	16.4 ± 0.3
Hematocrit (%)	48.0 ± 1.1	49.4 ± 1.1
Mean Cell Volume (μm^3)	51 ± 0	49 ± 0*
Mean Cell Hemoglobin (pg)	17.2 ± 0.1	16.2 ± 0.1*
Mean Cell Hemoglobin Concentration (g/dl)	33.7 ± 0.1	33.2 ± 0.1
Platelets ($10^3/\mu l$)	588 ± 32	613 ± 32
Cholesterol (mg/dl)	90 ± 2	66 ± 2*
Triglycerides (mg/dl)	111 ± 4	77 ± 4*
Blood Urea Nitrogen (mg/dl)	18.6 ± 1.1	19.1 ± 1.1
Creatinine (mg/dl)	0.6 ± 0.0	0.7 ± 0.0*
Glucose (mg/dl)	124 ± 7	105 ± 7
Total Protein (g/dl)	6.9 ± 0.1	6.8 ± 0.1
Albumin (g/dl)	5.0 ± 0.1	4.5 ± 0.1*
Alanine Aminotransferase (U/l)	47 ± 2	51.17 ± 2.01
Aspartate Aminotransferase (U/l))	74 ± 4	80 ± 4
Calcium (mg/dl)	9.2 ± 0.2	8.8 ± 0.2
Inorganic Phosphorus (mg/dl)	8.3 ± 0.6	8.0 ± 0.7
Sodium (mmol/l)	160 ± 0	158 ± 0*
Potassium (mmol/l)	6.7 ± 0.2	6.6 ± 0.2
Chloride (mmol/l)	107 ± 0	106 ± 0
Female		
Leukocyte Cell Count ($10^3/\mu l$)	6.7 ± 1.0	14.0 ± 1.1*
Neutrophils (%)	8.0 ± 2.8	19.0 ± 3.2*
Lymphocytes (%)	91.3 ± 2.7	79.9 ± 3.1*
Monocytes (%)	0.3 ± 0.2	0.6 ± 0.2
Eosinophils (%)	0.4 ± 0.2	0.4 ± 0.2
Basophils (%)	0.0 ± 0.0	0.0 ± 0.0
Erythrocyte Cell Count ($10^3/\mu l$)	9.04 ± 0.12	9.48 ± 0.13*
Hemoglobin (g/dl)	16.8 ± 0.2	15.7 ± 0.2*
Hematocrit (%)	48.1 ± 0.6	47.2 ± 0.7
Mean Cell Volume (μm^3)	53.± 0	50 ± 0*
Mean Cell Hemoglobin (pg)	18.5 ± 0.1	16.5 ± 0.1*
Mean Cell Hemoglobin Concentration (g/dl)	34.8 ± 0.1	33.2 ± 0.2
Platelets ($10^3/\mu l$)	579 ± 24	740 ± 28*
Cholesterol (mg/dl)	111 ± 3	79 ± 4*
Triglycerides (mg/dl)	76 ± 11	103 ± 13
Blood Urea Nitrogen (mg/dl)	17.1 ± 0.8	20.8 ± 0.9*
Creatinine (mg/dl)	0.6 ± 0.0	0.7 ± 0.0
Glucose (mg/dl)	83 ± 2	78 ± 2
Total Protein (g/dl)	6.8 ± 0.1	6.1 ± 0.1*
Albumin (g/dl)	4.9 ± 0.1	3.8 ± 0.1*
Alanine Aminotransferase (U/l)	45 ± 3	47 ± 3
Aspartate Aminotransferase (U/l))	82 ± 3	79 ± 3
Calcium (mg/dl)	9.9 ± 0.2	8.9 ± 0.2*
Inorganic Phosphorus (mg/dl)	7.9 ± 0.2	8.0 ± 0.2
Sodium (mmol/l)	159 ± 1	154 ± 1*
Potassium (mmol/l)	6.4 ± 0.1	6.8 ± 0.1*
Chloride (mmol/l)	107 ± 1	104 ± 1*

[a] Values are given as LS means ± standard error of the mean; n=24 male rats (12/group) except n=23 male rats for Inorganic phorphorus; n=21 female rats (12 controls and 9 dosed).
[b] Significance at P≤0.05 is indicated by "*" and for the dosed group represents comparison to the control group based on Dunnett's test.

TABLE E3
Urinalysis Data for Rats in the 14-Day Drinking Water Study of Aloe vera Extracts[a]

	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
Gel Extract						
Week 1						
Male						
Volume (ml/24h)	5.1 ± 0.7	4.7 ± 0.7	5.3 ± 0.7	4.6 ± 0.7	4.8 ± 0.7	5.1 ± 0.7
Urine Creatinine (mg/dl)	119.8 ± 11.9	109.8 ± 11.9	85.8 ± 11.9	115.8 ± 11.9	110.0 ± 11.9	113.0 ± 11.9
Micro Protein (mg/dl)	70.7 ± 12.0	55.3 ± 12.0	39.0 ± 12.0	58.4 ± 12.0	59.3 ± 12.0	51.0 ± 12.0
Urine Glucose (mg/dl)	77.3 ± 11.1	64.0 ± 11.1	47.5 ± 11.1	83.3 ± 11.1	72.0 ± 11.1	79.3 ± 11.1
Urine Creatinine (mg/24h)	6.1 ± 0.5	5.2 ± 0.5	4.6 ± 0.5	5.2 ± 0.5	5.0 ± 0.5	5.0 ± 0.5
Micro Protein (mg/24hl)	3.5 ± 0.7	2.6 ± 0.7	2.2 ± 0.7	2.7 ± 0.7	3.1 ± 0.7	2.6 ± 0.7
Urine Glucose (mg/24h)	3.9 ± 0.7	3.0 ± 0.7	2.6 ± 0.7	3.7 ± 0.7	3.5 ± 0.7	4.1 ± 0.7
Female						
Volume (ml/24h)	5.8 ± 1.2	6.4 ± 1.2	6.3 ± 1.2	5.7 ± 1.2	8.2 ± 1.2	4.7 ± 1.2
Urine Creatinine (mg/dl)	90.8 ± 11.5	86.0 ± 11.5	72.5 ± 11.5	83.5 ± 11.5	77.5 ± 11.5	105.8 ± 11.5
Micro Protein (mg/dl)	24.0 ± 3.3	22.8 ± 3.3	20.6 ± 3.3	21.8 ± 3.3	18.3 ± 3.3	27.4 ± 3.3
Urine Glucose (mg/dl)	50.3 ± 5.6*	33.5 ± 5.6	41.0 ± 5.6	43.0 ± 5.6	39.3 ± 5.6	62.3 ± 5.6
Urine Creatinine (mg/24h)	5.0 ± 0.3	5.4 ± 0.3	4.5 ± 0.3	4.7 ± 0.3	5.0 ± 0.3	4.6 ± 0.3
Micro Protein (mg/24hl)	1.3 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	1.2 ± 0.2
Urine Glucose (mg/24h)	2.8 ± 0.4	2.1 ± 0.4	2.6 ± 0.4	2.4 ± 0.4	2.9 ± 0.4	2.9 ± 0.4
Week 2						
Male						
Volume (ml/24h)	6.7 ± 0.6	6.2 ± 0.6	5.9 ± 0.6	5.9 ± 0.6	5.7 ± 0.6	6.3 ± 0.6
Urine Creatinine (mg/dl)	136.3 ± 11.7	130.5 ± 11.7	125.3 ± 11.7	126.3 ± 11.7	120.0 ± 11.7	114.0 ± 11.7
Micro Protein (mg/dl)	93.7 ± 9.7	91.6 ± 9.7	92.9 ± 9.7	96.3 ± 9.7	96.6 ± 9.7	95.3 ± 9.7
Urine Glucose (mg/dl)	58.5 ± 6.2	55.5 ± 6.2	62.8 ± 6.2	64.0 ± 6.2	65.5 ± 6.2	68.3 ± 6.2
Urine Creatinine (mg/24h)	8.5 ± 0.5*	8.0 ± 0.5	7.4 ± 0.5	7.5 ± 0.5	6.9 ± 0.5	6.7 ± 0.5
Micro Protein (mg/24hl)	5.9 ± 0.6	5.5 ± 0.6	5.5 ± 0.6	5.6 ± 0.6	5.6 ± 0.6	6.0 ± 0.6
Urine Glucose (mg/24h)	3.7 ± 0.6	3.5 ± 0.6	3.7 ± 0.6	3.8 ± 0.6	3.8 ± 0.6	4.3 ± 0.6
Female						
Volume (ml/24h)	6.8 ± 1.4	7.5 ± 1.4	7.7 ± 1.4	9.0 ± 1.4	8.7 ± 1.4	6.9 ± 1.4
Urine Creatinine (mg/dl)	89.0 ± 12.8	82.5 ± 12.8	83.0 ± 12.8	80.3 ± 12.8	65.8 ± 12.8	102.5 ± 12.8
Micro Protein (mg/dl)	30.0 ± 6.6	24.0 ± 6.6	27.2 ± 6.6	21.5 ± 6.6	24.3 ± 6.6	43.2 ± 6.6
Urine Glucose (mg/dl)	39.3 ± 9.5*	34.8 ± 9.5	39.3 ± 9.5	33.3 ± 9.5	42.5 ± 9.5	71.3 ± 9.5
Urine Creatinine (mg/24h)	5.9 ± 0.4	6.1 ± 0.4	6.2 ± 0.4	6.0 ± 0.4	5.1 ± 0.4	6.3 ± 0.4
Micro Protein (mg/24hl)	1.9 ± 0.5	1.8 ± 0.5	2.0 ± 0.5	1.6 ± 0.5	1.9 ± 0.5	2.9 ± 0.5
Urine Glucose (mg/24h)	2.5 ± 0.7*	2.6 ± 0.7	3.0 ± 0.7	2.6 ± 0.7	3.5 ± 0.7	4.8 ± 0.7
Decolorized Whole Leaf Extract						
Week 1						
Male						
Volume (ml/24h)	5.5 ± 0.3	5.0 ± 0.3	5.6 ± 0.3	5.3 ± 0.3	4.8 ± 0.3	5.8 ± 0.3
Urine Creatinine (mg/dl)	135.3 ± 6.1	133.3 ± 6.1	126.3 ± 6.1	144.8 ± 6.1	154.0 ± 6.1	132.8 ± 6.1
Micro Protein (mg/dl)	69.1 ± 12.2	69.0 ± 12.2	71.7 ± 12.2	78.0 ± 12.2	69.3 ± 12.2	63.6 ± 12.2
Urine Glucose (mg/dl)	120.0 ± 22.6	163.5 ± 22.6	149.0 ± 22.6	131.0 ± 22.6	124.3 ± 22.6	149.3 ± 22.6
Urine Creatinine (mg/24h)	7.4 ± 0.4	6.7 ± 0.4	7.0 ± 0.4	7.7 ± 0.4	7.3 ± 0.4	7.6 ± 0.4
Micro Protein (mg/24hl)	3.8 ± 0.7	3.4 ± 0.7	3.9 ± 0.7	4.1 ± 0.7	3.4 ± 0.7	3.7 ± 0.7
Urine Glucose (mg/24h)	6.7 ± 1.3	8.2 ± 1.3	8.1 ± 1.3	7.0 ± 1.3	5.9 ± 1.3	8.7 ± 1.3
Female						
Volume (ml/24h)	6.3 ± 0.7	5.8 ± 0.7	5.2 ± 0.7	4.2 ± 0.7	5.0 ± 0.7	5.9 ± 0.7
Urine Creatinine (mg/dl)	102.8 ± 8.6	108.3 ± 8.6	111.5 ± 8.6	127.3 ± 8.6	123.0 ± 8.6	114.5 ± 8.6
Micro Protein (mg/dl)	25.9 ± 3.6	26.8 ± 3.6	32.1 ± 3.6	33.0 ± 3.6	40.8 ± 3.6*	28.6 ± 3.6
Urine Glucose (mg/dl)	107.5 ± 23.9	135.8 ± 23.9	127.5 ± 23.9	112.3 ± 23.9	129.8 ± 23.9	108.0 ± 23.9
Urine Creatinine (mg/24h)	6.4 ± 0.4	6.0 ± 0.4	5.6 ± 0.4	5.3 ± 0.4	6.1 ± 0.4	6.4 ± 0.4
Micro Protein (mg/24hl)	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.4 ± 0.2	2.0 ± 0.2	1.6 ± 0.2
Urine Glucose (mg/24h)	6.7 ± 0.8	7.8 ± 0.8	5.8 ± 0.8	4.8 ± 0.8	6.2 ± 0.8	5.6 ± 0.8

TABLE E3
Urinalysis Data for Rats in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
Decolorized Whole Leaf Extract (continued)						
Week 2						
Male						
Volume (ml/24h)	7.1 ± 1.1	6.2 ± 1.1	9.2 ± 1.1	6.4 ± 1.1	5.4 ± 1.1	7.4 ± 1.1
Urine Creatinine (mg/dl)	143.0 ± 15.3	151.8 ± 13.2	108.5 ± 13.2	145.0 ± 13.2	157.5 ± 13.2	133.8 ± 13.2
Micro Protein (mg/dl)	114.0 ± 13.5	120.1 ± 11.7	79.6 ± 11.7	119.2 ± 11.7	120.3 ± 11.7	98.5 ± 11.7
Urine Glucose (mg/dl)	96.7 ± 15.8	106.8 ± 13.7	78.3 ± 13.7	104.5 ± 13.7	116.0 ± 13.7	86.8 ± 13.7
Urine Creatinine (mg/24h)	7.5 ± 1.2	9.2 ± 1.2	8.2 ± 1.2	9.2 ± 1.2	8.5 ± 1.2	9.7 ± 1.2
Micro Protein (mg/24hl)	6.0 ± 1.3	7.3 ± 1.3	6.9 ± 1.3	7.6 ± 1.3	6.6 ± 1.3	7.4 ± 1.3
Urine Glucose (mg/24h)	5.0 ± 1.0	6.4 ± 1.0	6.0 ± 1.0	6.6 ± 1.0	6.3 ± 1.0	6.5 ± 1.0
Female						
Volume (ml/24h)	6.8 ± 0.9	9.3 ± 0.9	7.9 ± 0.9	7.2 ± 0.9	7.7 ± 0.9	6.8 ± 0.9
Urine Creatinine (mg/dl)	115.3 ± 9.7	92.5 ± 9.7	104.0 ± 9.7	116.5 ± 9.7	103.0 ± 9.7	111.8 ± 9.7
Micro Protein (mg/dl)	45.5 ± 12.8	25.8 ± 12.8	27.2 ± 12.8	56.9 ± 12.8	37.6 ± 12.8	31.1 ± 12.8
Urine Glucose (mg/dl)	63.8 ± 10.0	45.5 ± 10.0	45.0 ± 10.0	73.3 ± 10.0	56.3 ± 10.0	56.5 ± 10.0
Urine Creatinine (mg/24h)	7.8 ± 0.5	8.2 ± 0.5	8.2 ± 0.5	8.0 ± 0.5	7.5 ± 0.5	7.6 ± 0.5
Micro Protein (mg/24hl)	3.0 ± 0.8	2.3 ± 0.8	2.2 ± 0.8	4.0 ± 0.8	2.7 ± 0.8	2.1 ± 0.8
Urine Glucose (mg/24h)	4.3 ± 0.6	4.0 ± 0.6	3.5 ± 0.6	5.2 ± 0.6	3.9 ± 0.6	3.8 ± 0.6
Whole Leaf Extract						
Week 1						
Male						
Volume (ml/24h)	4.7 ± 0.5*	4.8 ± 0.5	3.0 ± 0.5	2.0 ± 0.5*	1.6 ± 0.5*	1.4 ± 0.5*
Urine Creatinine (mg/dl)	125.8 ± 20.2*	129.5 ± 20.2	169.0 ± 20.2	163.8 ± 20.2	185.3 ± 20.2	220.0 ± 20.2*
Micro Protein (mg/dl)	56.6 ± 11.6	64.5 ± 11.6	68.7 ± 11.6	56.6 ± 11.6	54.5 ± 11.6	85.0 ± 11.6
Urine Glucose (mg/dl)	85.8 ± 98.9	109.8 ± 98.9	390.0 ± 98.9	224.8 ± 98.9	100.3 ± 98.9	262.0 ± 98.9
Urine Creatinine (mg/24h)	5.9 ± 0.8*	6.0 ± 0.8	4.9 ± 0.8	3.4 ± 0.8	3.2 ± 0.8	3.1 ± 0.8
Micro Protein (mg/24hl)	2.8 ± 0.6*	3.2 ± 0.6	1.8 ± 0.6	1.2 ± 0.6	0.9 ± 0.6	1.2 ± 0.6
Urine Glucose (mg/24h)	4.1 ± 1.7	5.3 ± 1.7	7.4 ± 1.7	5.3 ± 1.7	1.7 ± 1.7	3.7 ± 1.7
Female						
Volume (ml/24h)	5.0 ± 0.7*	3.9 ± 0.7	3.2 ± 0.7	3.4 ± 0.7	2.1 ± 0.7*	1.9 ± 0.7*
Urine Creatinine (mg/dl)	81.8 ± 11.1*	121.3 ± 11.1	161.3 ± 11.1*	177.8 ± 11.1*	155.0 ± 11.1*	177.5 ± 11.1*
Micro Protein (mg/dl)	32.8 ± 11.3	38.1 ± 11.3	62.0 ± 11.3	71.6 ± 11.3	48.9 ± 11.3	52.4 ± 11.3
Urine Glucose (mg/dl)	72.0 ± 60.1*	69.0 ± 60.1	97.8 ± 60.1	142.8 ± 60.1	123.5 ± 60.1	250.5 ± 60.1
Urine Creatinine (mg/24h)	4.0 ± 0.6	4.5 ± 0.6	5.0 ± 0.6	5.9 ± 0.6	3.2 ± 0.6	3.2 ± 0.6
Micro Protein (mg/24hl)	1.4 ± 0.3	1.3 ± 0.3	1.9 ± 0.3	2.3 ± 0.3	1.0 ± 0.3	0.9 ± 0.3
Urine Glucose (mg/24h)	2.9 ± 0.7	2.6 ± 0.7	3.0 ± 0.7	4.7 ± 0.7	2.5 ± 0.7	2.9 ± 0.7
Week 2						
Male						
Volume (ml/24h)	5.7 ± 0.4*	4.9 ± 0.4	3.2 ± 0.4*	3.2 ± 0.4*	2.5 ± 0.4*	2.6 ± 0.4*
Urine Creatinine (mg/dl)	134.8 ± 9.9*	138.0 ± 9.9	157.5 ± 9.9	176.5 ± 9.9*	169.0 ± 9.9	176.8 ± 9.9*
Micro Protein (mg/dl)	105.2 ± 11.3*	107.0 ± 11.3	109.4 ± 11.3	108.8 ± 11.3	69.3 ± 11.3	87.4 ± 11.3
Urine Glucose (mg/dl)	70.5 ± 23.4	100.3 ± 23.4	109.8 ± 23.4	92.3 ± 23.4	95.3 ± 23.4	129.5 ± 23.4
Urine Creatinine (mg/24h)	7.7 ± 0.5*	6.6 ± 0.5	5.1 ± 0.5*	5.7 ± 0.5	4.2 ± 0.5*	4.6 ± 0.5*
Micro Protein (mg/24hl)	6.0 ± 0.5*	5.2 ± 0.5	3.5 ± 0.5*	3.5 ± 0.5*	1.7 ± 0.5*	2.3 ± 0.5*
Urine Glucose (mg/24h)	4.0 ± 0.6	4.7 ± 0.6	3.4 ± 0.6	2.8 ± 0.6	2.3 ± 0.6	3.4 ± 0.6
Female						
Volume (ml/24h)	4.8 ± 0.5*	4.3 ± 0.5	3.2 ± 0.5	2.0 ± 0.5*	2.2 ± 0.5*	2.7 ± 0.5*
Urine Creatinine (mg/dl)	95.3 ± 11.8*	129.0 ± 11.8	153.5 ± 11.8*	204.5 ± 11.8*	163.3 ± 11.8*	163.5 ± 11.8*
Micro Protein (mg/dl)	44.5 ± 6.7	40.6 ± 6.7	44.4 ± 6.7	45.3 ± 6.7	42.6 ± 6.7	49.2 ± 6.7
Urine Glucose (mg/dl)	49.0 ± 13.2	64.3 ± 13.2	72.8 ± 13.2	76.3 ± 13.2	67.3 ± 13.2	86.3 ± 13.2
Urine Creatinine (mg/24h)	4.6 ± 0.6	5.5 ± 0.6	4.8 ± 0.6	3.9 ± 0.6	3.6 ± 0.6	4.2 ± 0.6
Micro Protein (mg/24hl)	1.9 ± 0.2*	1.8 ± 0.2	1.4 ± 0.2	0.9 ± 0.2*	0.9 ± 0.2*	1.3 ± 0.2
Urine Glucose (mg/24h)	2.4 ± 0.5	2.7 ± 0.5	2.1 ± 0.5	1.4 ± 0.5	1.4 ± 0.5	2.4 ± 0.5

[a] Values are given as LS means ± standard error of the mean (n=4/group). Significance at P≤0.05 is indicated by "*", which represents the test for linear trend under the 0% group and represents comparison to the control group based on Dunnett's test under the dosed groups.

TABLE E4
Urinalysis Data for Rats in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract[a]

	0%	2%
Day 30		
Male		
Total Volume (ml/24 h)	5.9 ± 0.4[a]	3.5 ± 0.4*
Urine Creatinine (mg/dl)	218.3 ± 24.8	198.7 ± 24.8
Micro Protein (mg/dl)	121.0 ± 17.1	188.8 ± 17.1*
Urine Glucose (mg/dl)	78.3 ± 0.9	77.3 ± 0.9
Urine Creatinine (mg/24 h)	13.6 ± 2.2	7.0 ± 2.2*
Micro Protein (mg/24 h)	7.3 ± 1.0	6.2 ± 1.0
Urine Glucose (mg/24 h)	4.6 ± 0.3	2.7 ± 0.3*
Female		
Total Volume (ml/24 h)	8.4 ± 0.8	2.0 ± 0.9*
Urine Creatinine (mg/dl)	112.5 ± 14.6	202.3 ± 16.9*
Micro Protein (mg/dl)	45.3 ± 35.4	250.2 ± 40.8*
Urine Glucose (mg/dl)	27.0 ± 3.0	53.2 ± 3.4*
Urine Creatinine (mg/24 h)	8.3 ± 0.6	3.8 ± 0.7*
Micro Protein (mg/24 h)	3.4 ± 0.7	4.5 ± 0.8
Urine Glucose (mg/24 h)	2.0 ± 0.2	1.0 ± 0.2*
Day 60		
Male		
Total Volume (ml/24 h)	3.8 ± 0.3	2.8 ± 0.3*
Urine Creatinine (mg/dl)	238.9 ± 15.0	211.1 ± 15.0
Micro Protein (mg/dl)	51.4 ± 12.6	87.3 ± 12.6*
Urine Glucose (mg/dl)	76.6 ± 1.0	76.1 ± 1.0
Urine Creatinine (mg/24 h)	8.4 ± 0.4	5.8 ± 0.4*
Micro Protein (mg/24 h)	1.8 ± 0.4	2.5 ± 0.4
Urine Glucose (mg/24 h)	2.8 ± 0.2	2.1 ± 0.2*
Female		
Total Volume (ml/24 h)	6.6 ± 0.4	2.2 ± 0.5*
Urine Creatinine (mg/dl)	116.6 ± 10.4	166.9 ± 12.0*
Micro Protein (mg/dl)	97.0 ± 19.6	117.7 ± 22.6
Urine Glucose (mg/dl)	21.8 ± 2.1	42.9 ± 2.5
Urine Creatinine (mg/24 h)	7.5 ± 0.3	3.6 ± 0.4*
Micro Protein (mg/24 h)	5.8 ± 0.8	2.5 ± 0.9*
Urine Glucose (mg/24h)	1.4 ± 0.1	0.9 ± 0.1*
Day 90		
Male		
Total Volume (ml/24 h)	3.5 ± 0.4	3.1 ± 0.4
Urine Creatinine (mg/dl)	277.9 ± 21.1	214.9 ± 21.1*
Micro Protein (mg/dl)	48.3 ± 7.1	47.7 ± 7.1
Urine Glucose (mg/dl)	81.5 ± 2.0	76.4 ± 2.0
Urine Creatinine (mg/24 h)	9.4 ± 0.8	6.0 ± 0.8*
Micro Protein (mg/24 h)	1.6 ± 0.2	1.2 ± 0.2
Urine Glucose (mg/24 h)	2.8 ± 0.3	2.3 ± 0.3
Female		
Total Volume (ml/24 h)	5.9 ± 0.6	2.8 ± 0.7*
Urine Creatinine (mg/dl)	141.3 ± 14.2	192.2 ± 16.4*
Micro Protein (mg/dl)	141.0 ± 44.2	132.7 ± 51.0
Urine Glucose (mg/dl)	27.2 ± 3.0	63.4 ± 3.5*
Urine Creatinine (mg/24 h)	7.9 ± 0.9	5.3 ± 1.0
Micro Protein (mg/24 h)	6.5 ± 1.2	4.0 ± 1.4
Urine Glucose (mg/24h)	1.5 ± 0.2	1.7 ± 0.3

[a] Values for the parameters tested are given as LS mean ± standard error of the mean (n=24 males; 12 /group or 21 females; 12 control and 9 dosed).
* Signifies values that are significantly different ($P \leq 0.05$) from the control group by Tukey's tests.

TABLE E5
Hematology and Clinical Chemistry Data for Mice in the 14-Day Drinking Water Study of Aloe vera Extracts[a]

	0%	0.5%	1.0%	1.5%	2.0%	3.0%
Gel Extract						
Male						
Leukocyte Cell Count ($10^3/\mu l$)[b]	2.5 ± 0.6	4.7 ± 0.6*	3.1 ± 0.6	1.7 ± 0.6	2.0 ± 0.6	2.2 ± 0.6
Erythrocyte Cell Count ($10^3/\mu l$)[b]	8.95 ± 0.33	9.52 ± 0.33	9.29 ± 0.33	9.53 ± 0.33	9.41 ± 0.33	9.34 ± 0.33
Hemoglobin (g/dl)[b]	17.2 ± 0.8	16.0 ± 0.8	18.9 ± 0.8	19.2 ± 0.8	15.9 ± 0.8	15.9 ± 0.8
Hematocrit (%)[b]	43.6 ± 1.5	46.7 ± 1.5	45.1 ± 1.5	45.8 ± 1.5	46.3 ± 1.5	45.8 ± 1.5
Mean Cell Volume (μm^3)[b]	49 ± 0	49 ± 0	49 ± 0	48 ± 0	49 ± 0	49 ± 0
Mean Cell Hemoglobin (pg)[b]	19.5 ± 1.1	16.9 ± 1.1	20.6 ± 1.1	20.3 ± 1.1	16.9 ± 1.1	17.0 ± 1.1
Mean Cell Hemoglobin Concentration (g/dl)[b]	40.1 ± 2.4	34.3 ± 2.4	42.5 ± 2.4	42.2 ± 2.4	34.3 ± 2.4	34.7 ± 2.4
Platelets ($10^3/\mu l$)[b]	780 ± 55	979 ± 55	849 ± 55	827 ± 55	841 ± 55	829 ± 55
Blood Urea Nitrogen (mg/dl)[j]	19.1 ± 1.3	17.6 ± 1.3	21.4 ± 1.3	25.0 ± 1.5*	17.0 ± 1.3	16.1 ± 1.3
Creatinine (mg/dl)[b]	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
Glucose (mg/dl)[k]	164 ± 12*	138 ± 12	162 ± 12	165 ± 14	117 ± 14	138 ± 12
Total Protein (g/dl)[d]	5.4 ± 0.2*	5.6 ± 0.2	5.8 ± 0.2	5.6 ± 0.2	5.8 ± 0.2	6.2 ± 0.2*
Albumin (g/dl)[f]	3.6 ± 0.1*	3.7 ± 0.1	3.8 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.1*
Alanine Aminotransferase (U/l)[i]	35 ± 3	28 ± 4	31 ± 4	26 ± 3	36 ± 4	28 ± 4
Aspartate Aminotransferase (U/l)[h]	67 ± 9	63 ± 9	60 ± 9	46 ± 9	59 ± 12	60 ± 9
Calcium (mg/dl)[b]	9.7 ± 0.2	9.9 ± 0.2	10.1 ± 0.2	10.1 ± 0.2	9.7 ± 0.2	10.2 ± 0.2
Inorganic Phosphorus (mg/dl)[f]	11.5 ± 0.8*	10.6 ± 0.7	10.8 ± 0.7	11.3 ± 0.9	11.1 ± 0.8	10.9 ± 0.7
Female						
Leukocyte Cell Count ($10^3/\mu l$)[c]	2.6 ± 0.9	7.0 ± 1.0*	6.9 ± 0.9*	2.4 ± 0.9	4.4 ± 0.9	4.2 ± 0.9
Erythrocyte Cell Count ($10^3/\mu l$)[c]	9.16 ± 0.27	9.63 ± 0.29	9.70 ± 0.27	9.40 ± 0.27	9.50 ± 0.27	9.79 ± 0.27
Hemoglobin (g/dl)[c]	15.3 ± 0.4*	16.5 ± 0.4	16.4 ± 0.4	16.2 ± 0.4	16.3 ± 0.4	17.1 ± 0.4*
Hematocrit (%)[c]	45.7 ± 1.1	47.5 ± 1.1	48.9 ± 1.1	46.3 ± 1.1	47.0 ± 1.1	48.2 ± 1.1
Mean Cell Volume (μm^3)[c]	50 ± 0	49 ± 1	51 ± 0	49 ± 0	50 ± 0	49 ± 0
Mean Cell Hemoglobin (pg)[c]	16.6 ± 0.4	17.2 ± 0.4	16.9 ± 0.4	17.2 ± 0.4	17.3 ± 0.4	17.5 ± 0.4
Mean Cell Hemoglobin Concentration (g/dl)[c]	33.5 ± 0.6*	34.8 ± 0.7	33.5 ± 0.6	35.0 ± 0.6	34.8 ± 0.6	35.5 ± 0.6
Platelets ($10^3/\mu l$)[c]	827 ± 48	722 ± 52	815 ± 49	769 ± 48	805 ± 48	810 ± 48
Blood Urea Nitrogen (mg/dl)[k]	17.8 ± 1.0	21.1 ± 1.1	18.0 ± 1.1	21.7 ± 1.0*	18.3 ± 1.0	21.3 ± 1.0
Creatinine (mg/dl)[g]	0.7 ± 0.0	0.67 ± 0.04	0.70 ± 0.04	0.63 ± 0.04	0.63 ± 0.04	0.66 ± 0.04
Glucose (mg/dl)[m]	115 ± 7*	104 ± 8	108 ± 8	114 ± 7	94 ± 7	79 ± 8*
Total Protein (g/dl)[g]	5.7 ± 0.2*	5.8 ± 0.2	6.0 ± 0.2	5.8 ± 0.2	6.0 ± 0.2	6.2 ± 0.2
Albumin (g/dl)[n]	4.6 ± 0.1*	4.2 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.8 ± 0.1	4.8 ± 0.2
Alanine Aminotransferase (U/l)[o]	35 ± 5	37 ± 8	25 ± 8	33 ± 5	44 ± 7	37 ± 12
Aspartate Aminotransferase (U/l)[p]	51 ± 16	68 ± 20	56 ± 28	57 ± 13	88 ± 28	45 ± 28
Calcium (mg/dl)[f]	10.0 ± 0.3	10.2 ± 0.3	10.5 ± 0.4	10.2 ± 0.3	10.6 ± 0.3	10.3 ± 0.3
Inorganic Phosphorus (mg/dl)[l]	9.9 ± 0.6	13.0 ± 0.7*	11.1 ± 0.7	12.1 ± 0.6	12.5 ± 0.6*	12.4 ± 0.8

TABLE E5
Hematology and Clinical Chemistry Data for Mice in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

	0%	0.5%	1.0%	1.5%	2.0%	3.0%
Decolorized Whole Leaf Extract						
Male						
Leukocyte Cell Count ($10^3/\mu l$)[b]	4.2 ± 0.7	3.3 ± 0.7	2.7 ± 0.7	3.3 ± 0.7	2.8 ± 0.7	4.6 ± 0.7
Erythrocyte Cell Count ($10^3/\mu l$)[b]	8.55 ± 0.31	9.96 ± 0.31*	9.63 ± 0.31	9.49 ± 0.31	9.68 ± 0.31	9.51 ± 0.31
Hemoglobin (g/dl)[b]	14.7 ± 1.0	19.5 ± 1.0*	19.1 ± 1.0*	18.9 ± 1.0*	19.1 ± 1.0*	16.5 ± 1.0
Hematocrit (%)[b]	42.5 ± 1.3	47.9 ± 1.3*	46.5 ± 1.3	46 ± 1.3	46.9 ± 1.3	46.8 ± 1.3
Mean Cell Volume (μm^3)[b]	50 ± 1	48 ± 1*	48 ± 1	49 ± 1	48 ± 1	49 ± 1
Mean Cell Hemoglobin (pg)[b]	17.3 ± 1.1	19.7 ± 1.1	20.0 ± 1.1	20.0 ± 1.1	19.8 ± 1.1	17.3 ± 1.1
Mean Cell Hemoglobin Concentration (g/dl)[b]	34.5 ± 2.4	40.9 ± 2.4	41.5 ± 2.4	41.3 ± 2.4	41.0 ± 2.4	35.2 ± 2.4
Platelets ($10^3/\mu l$)[b]	879 ± 53	976 ± 53	863 ± 53	872 ± 53	967 ± 53	1009 ± 53
Blood Urea Nitrogen (mg/dl)[j]	24.5 ± 2.3	20.0 ± 2.3	22.9 ± 2.3	18.2 ± 2.3	23.3 ± 2.3	17.3 ± 2.6
Creatinine (mg/dl)[c]	0.6 ± 0.1	0.76 ± 0.06*	0.76 ± 0.06*	0.76 ± 0.06*	0.78 ± 0.06*	0.54 ± 0.06
Glucose (mg/dl)[k]	117 ± 9*	166 ± 9*	136 ± 9	156 ± 11	131 ± 9	110 ± 11
Total Protein (g/dl)[c]	5.9 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	6.3 ± 0.1	5.8 ± 0.1
Albumin (g/dl)[f]	3.9 ± 0.2	4.0 ± 0.2	3.9 ± 0.2	3.9 ± 0.2	4.2 ± 0.2	3.9 ± 0.2
Alanine Aminotransferase (U/l)[s]	37 ± 4	30 ± 5	41 ± 4	35 ± 6	34 ± 5	34 ± 5
Aspartate Aminotransferase (U/l)[u]	67 ± 11	59 ± 9	101 ± 9	77 ± 9	76 ± 9	80 ± 9
Calcium (mg/dl)[c]	10.1 ± 0.2	10.2 ± 0.2	10.0 ± 0.2	10.2 ± 0.2	10.1 ± 0.2	10.2 ± 0.2
Inorganic Phosphorus (mg/dl)[d]	11.2 ± 1.2*	11.2 ± 1.2	10.9 ± 1.2	12.7 ± 1.3	11.4 ± 1.2	14.0 ± 1.3
Female						
Leukocyte Cell Count ($10^3/\mu l$)[c]	3.4 ± 0.7*	4.1 ± 0.8	3.9 ± 0.7	4.1 ± 0.7	7.2 ± 0.7*	4.8 ± 0.7
Erythrocyte Cell Count ($10^3/\mu l$)[c]	9.56 ± 0.14*	9.84 ± 0.15	9.85 ± 0.14	9.85 ± 0.14	10.16 ± 0.14*	9.98 ± 0.14
Hemoglobin (g/dl)[c]	16.1 ± 0.3*	17.3 ± 0.3	16.8 ± 0.3	16.9 ± 0.3	17.9 ± 0.3*	17.4 ± 0.3*
Hematocrit (%)[c]	48.0 ± 0.6*	48.5 ± 0.7	49.7 ± 0.6	49.8 ± 0.6	50.4 ± 0.6*	49.3 ± 0.6
Mean Cell Volume (μm^3)[c]	50 ± 0	49 ± 1	51 ± 0	51 ± 0	50 ± 0	50 ± 0
Mean Cell Hemoglobin (pg)[c]	16.9 ± 0.4	17.5 ± 0.4	17.1 ± 0.4	17.1 ± 0.4	17.6 ± 0.4	17.4 ± 0.4
Mean Cell Hemoglobin Concentration (g/dl)[c]	33.6 ± 0.5	35.6 ± 0.6*	33.8 ± 0.5	33.9 ± 0.5	35.5 ± 0.5	35.3 ± 0.5
Platelets ($10^3/\mu l$)[c]	721 ± 56*	879 ± 60	827 ± 56	811 ± 56	872 ± 56	922 ± 56
Blood Urea Nitrogen (mg/dl)[o]	21.1 ± 2.4	20.3 ± 1.9	18.4 ± 1.7	19.4 ± 1.7	22.4 ± 1.9	23.6 ± 1.7
Creatinine (mg/dl)[n]	0.8 ± 0.2	1.0 ± 0.2	0.7 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	0.6 ± 0.2
Glucose (mg/dl)[q]	118 ± 15	72 ± 21	121 ± 10	138 ± 10	101 ± 15	108 ± 10
Total Protein (g/dl)[r]	5.9 ± 0.2	6.8 ± 0.2*	6.2 ± 0.2	6.3 ± 0.2	6.4 ± 0.2	6.0 ± 0.2
Albumin (g/dl)[s]	4.3 ± 0.2	5.1 ± 0.2*	4.7 ± 0.2	4.9 ± 0.2	5.1 ± 0.2*	4.8 ± 0.2
Alanine Aminotransferase (U/l)[t]	39 ± 7	39 ± 7	30 ± 6	32 ± 7	37 ± 11	43 ± 7
Aspartate Aminotransferase (U/l)[k]	55 ± 19	115 ± 23	76 ± 12	64 ± 16	84 ± 19	77 ± 19
Calcium (mg/dl)[d]	9.9 ± 0.2	10.6 ± 0.2	10.5 ± 0.2	10.8 ± 0.2*	10.5 ± 0.2	10.2 ± 0.2
Inorganic Phosphorus (mg/dl)[g]	16.1 ± 2.4*	16.9 ± 2.2	12.3 ± 1.9	11.1 ± 2.0	12.4 ± 2.2	11.7 ± 1.9

TABLE E5
Hematology and Clinical Chemistry Data for Mice in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

	0%	0.5%	1.0%	1.5%	2.0%	3.0%
Whole Leaf Extract						
Male						
Leukocyte Cell Count ($10^3/\mu l$)[d]	2.1 ± 0.5*	1.9 ± 0.4	3.5 ± 0.4	1.5 ± 0.4	2.5 ± 0.4	5.5 ± 0.4*
Erythrocyte Cell Count ($10^3/\mu l$)[d]	10.03 ± 0.72	9.5 ± 0.6	9.8 ± 0.6	6.7 ± 0.6*	9.6 ± 0.6	9.1 ± 0.6
Hemoglobin (g/dl)[d]	17.0 ± 1.5	19.5 ± 1.3	16.9 ± 1.3	11.8 ± 1.3*	16.7 ± 1.3	18.4 ± 1.3
Hematocrit (%)[d]	49.3 ± 3.6	46.4 ± 3.1	47.9 ± 3.1	32.8 ± 3.1*	47.2 ± 3.1	44.8 ± 3.1
Mean Cell Volume (μm^3)[d]	49 ± 0	49 ± 0	49 ± 0	49 ± 0	49 ± 0	50 ± 0
Mean Cell Hemoglobin (pg)[d]	17.0 ± 1.1	20.6 ± 1.0	17.3 ± 1.0	17.3 ± 1.0	17.4 ± 1.0	20.5 ± 1.0
Mean Cell Hemoglobin Concentration (g/dl)[d]	34.6 ± 2.3	42.3 ± 2.0	35.3 ± 2.0	35.6 ± 2.0	35.5 ± 2.0	41.5 ± 2.0
Platelets ($10^3/\mu l$)[d]	1004 ± 84	928 ± 73	903 ± 73	754 ± 73	1000 ± 73	929 ± 73
Blood Urea Nitrogen (mg/dl)[j]	20.4 ± 4.0	21.4 ± 3.5	19.5 ± 3.5	18.0 ± 3.5	20.7 ± 3.5	22.0 ± 3.5
Creatinine (mg/dl)[c]	0.5 ± 0.1*	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.1*
Glucose (mg/dl)[j]	134 ± 12	149 ± 10	105 ± 10	108 ± 10	98 ± 10	160 ± 10
Total Protein (g/dl)[c]	6.0 ± 0.2	5.9 ± 0.2	6.1 ± 0.2	6.0 ± 0.2	6.0 ± 0.2	5.7 ± 0.2
Albumin (g/dl)[e]	3.9 ± 0.1*	3.9 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.7 ± 0.1
Alanine Aminotransferase (U/l)[g]	37 ± 6	40 ± 5	30 ± 5	35 ± 5	44 ± 6	33± 6
Aspartate Aminotransferase (U/l)[f]	73 ± 11	67 ± 8	71 ± 8	71 ± 8	72 ± 8	57 ± 9
Calcium (mg/dl)[c]	10.0 ± 0.2	10.0 ± 0.2	10.0 ± 0.2	10.0 ± 0.2	10.4 ± 0.2	10.4 ± 0.2
Inorganic Phosphorus (mg/dl)[e]	11 ± 0.6*	10.1 ± 0.6	10.0 ± 0.6	11.8 ± 0.6	12.2 ± 0.6	12.1 ± 0.6
Female						
Leukocyte Cell Count ($10^3/\mu l$)[b]	2.8 ± 0.6	4.0 ± 0.6	3.3 ± 0.6	3.7 ± 0.7	2.6 ± 0.6	2.4 ± 0.6
Erythrocyte Cell Count ($10^3/\mu l$)[b]	9.72 ± 0.30	9.48 ± 0.30	9.79 ± 0.30	9.92 ± 0.32	9.69 ± 0.30	9.61 ± 0.30
Hemoglobin (g/dl)[b]	17.0 ± 0.5	16.0 ± 0.5	16.6 ± 0.5	17.8 ± 0.6	16.4 ± 0.5	16.3 ± 0.5
Hematocrit (%)[b]	48.3 ± 1.3	47.7 ± 1.3	49.3 ± 1.3	49.4 ± 1.4	48.9 ± 1.3	48.1 ± 1.3
Mean Cell Volume (μm^3)[b]	50 ± 0.5	51 ± 1	51 ± 1	50 ± 1	51 ± 1	50 ± 1
Mean Cell Hemoglobin (pg)[b]	17.5 ± 0.3	16.84± 0.3	17.0 ± 0.3	17.9 ± 0.4	16.9 ± 0.3	16.9 ± 0.3
Mean Cell Hemoglobin Concentration (g/dl)[b]	35.3 ± 0.5	33.4 ± 0.5*	33.7 ± 0.5	35.9 ± 0.5	33.5 ± 0.5*	33.8 ± 0.5
Platelets ($10^3/\mu l$)[b]	832 ± 40	797 ± 40	828 ± 40	930 ± 43	806 ± 40	851 ± 40
Blood Urea Nitrogen (mg/dl)[i]	21.3 ± 1.1	17.9 ± 1.1	18.8 ± 1.3	21.4 ± 1.3	16.8 ± 1.1*	18.5 ± 1.1
Creatinine (mg/dl)[b]	0.7 ± 0.1	0.71 ± 0.04	0.67 ± 0.05	0.61± 0.05	0.79 ± 0.04	0.61 ± 0.04
Glucose (mg/dl)[k]	89 ± 7*	108 ± 7	136 ± 8*	101 ± 8	139 ± 7*	134 ± 7*
Total Protein (g/dl)[d]	6.2 ± 0.2	6.0± 0.2	6.1 ± 0.2	6.1 ± 0.2	6.1 ± 0.2	6.2 ± 0.2
Albumin (g/dl)[f]	4.6 ± 0.2	4.4 ± 0.2	4.6 ± 0.2	4.3 ± 0.2	4.6 ± 0.2	4.6 ± 0.2
Alanine Aminotransferase (U/l)[i]	69 ± 10*	32 ± 7*	29 ± 8*	36 ± 8*	35 ± 7*	29 ± 8*
Aspartate Aminotransferase (U/l)[h]	66 ± 11	59 ± 9	49 ± 8	53 ± 8	59 ± 7	61 ± 7
Calcium (mg/dl)[b]	10.2 ± 0.2	10.2 ± 0.2	10.1 ± 0.2	10.4 ± 0.2	10.4 ± 0.2	10.1 ± 0.2
Inorganic Phosphorus (mg/dl)[f]	11.9 ± 1.2	11.8 ± 1.2	12.0 ± 1.3	12.1 ± 1.3	15.4 ± 1.2	11.3 ± 1.2

TABLE E5
Hematology and Clinical Chemistry Data for Mice in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

[a] Values are given as LS means ± standard error of the mean. An asterisk (*) denotes significance at P ≤ 0.05; when listed under the 0% group, "*" represents the test for linear trend and when listed for the dosed groups, "*" represents comparison to the control group based on Dunnett's test.
[b] n=48 total
[c] n=47 total
[d] n=46 analyzed in total
[e] n=45 analyzed in total
[f] n=44 analyzed in total
[g] n=40 analyzed in total
[h] n=35 analyzed in total
[i] n=33 analyzed in total
[j] n=23 analyzed in total
[k] n=22 analyzed in total
[l] n=42 analyzed in total
[m] n=21 analyzed in total
[n] n=38 analyzed in total
[o] n=20 analyzed in total
[p] n=13 analyzed in total
[q] n=17 analyzed in total
[r] n=39 analyzed in total
[s] n=36 analyzed in total
[t] n=26 analyzed in total
[u] n=43 analyzed in total

Table E6
Hematology and Clinical Chemistry Data for Mice
in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract

	0%	3%
Male		
Leukocyte Cell Count ($10^3/\mu l$)[b]	2.8 ± 0.4[a]	2.8 ± 0.4
Neutrophils (%)[b]	2.6 ± 0.5	6.1 ± 0.5*
Lymphocytes (%)[b]	92.3 ± 1.3	88.2 ± 1.3*
Monocytes (%)[b]	4.2 ± 0.8	4.2 ± 0.8
Eosinophils (%)[b]	0.1 ± 0.1	0.2 ± 0.1
Basophils (%)[b]	0.8 ± 0.3	1.7 ± 0.3*
Erythrocyte Cell Count ($10^3/\mu l$)[b]	10.16 ± 0.10	10.07 ± 0.10
Hemoglobin (g/dl)[b]	16.2 ± 0.2	16.2 ± 0.2
Hematocrit (%)[b]	49.4 ± 0.6	49.3 ± 0.6
Mean Cell Volume (μm^3)[b]	49 ± 0	49 ± 0
Mean Cell Hemoglobin (pg)[b]	16.0 ± 0.1	16.1 ± 0.1
Mean Cell Hemoglobin Concentration (g/dl)[b]	32.9 ± 0.1	32.8 ± 0.1
Platelets ($10^3/\mu l$)[b]	877 ± 19	887 ± 19
Cholesterol (mg/dl)[d]	159 ± 4	152 ± 4
Triglycerides (mg/dl)[d]	120 ± 8	138 ± 7
Blood Urea Nitrogen (mg/dl)[b]	20.1 ± 0.4	20. ± 0.4
Creatinine (mg/dl)[b]	0.6 ± 0.0	0.8 ± 0.0*
Glucose (mg/dl)[b]	86 ± 4	103 ± 4*
Total Protein (g/dl)[b]	6.8 ± 0.2	6.8 ± 0.2
Albumin (g/dl)[b]	4.7 ± 0.1	4.5 ± 0.1*
Alanine Aminotransferase (U/l)[e]	39 ± 6	42 ± 6
Aspartate Aminotransferase (U/l)[b]	80 ± 13	90 ± 12
Calcium (mg/dl)[b]	10.7 ± 0.1	10.4 ± 0.1*
Inorganic Phosphorus (mg/dl)[b]	8.4 ± 0.2	8.6 ± 0.2
Female		
Leukocyte Cell Count ($10^3/\mu l$)[b]	3.4 ± 0.6	3.0 ± 0.6
Neutrophils (%)[b]	3.0 ± 0.4	3.5 ± 0.4
Lymphocytes (%[b]	93.1 ± 0.9	90.4 ± 0.9
Monocytes (%)[b]	3.7 ± 0.7	4.0 ± 0.7
Eosinophils (%)[b]	0.1 ± 0.0	0.1 ± 0.0
Basophils (%)a[b]	0.3 ± 0.2	2.0 ± 0.2*
Erythrocyte Cell Count ($10^3/\mu l$)[b]	10.33 ± 0.08	10.41 ± 0.08
Hemoglobin (g/dl)[b]	16.7 ± 0.1	17.0 ± 0.1
Hematocrit (%)[b]	50.5 ± 0.4	51.4 ± 0.4
Mean Cell Volume (μm^3)[b]	49 ± 0	49 ± 0
Mean Cell Hemoglobin (pg)[b]	16.2 ± 0.1	16.3 ± 0.1
Mean Cell Hemoglobin Concentration (g/dl)[b]	33.1 ± 0.1	33.0 ± 0.1
Platelets ($10^3/\mu l$)[b]	763 ± 20	754 ± 20
Cholesterol (mg/dl)[c]	120 ± 6	115 ± 6
Triglycerides (mg/dl)[c]	77 ± 6	74 ± 6
Blood Urea Nitrogen (mg/dl)[b]	18.7 ± 0.6	17.7 ± 0.6
Creatinine (mg/dl)[b]	0.5 ± 0.0	0.7 ± 0.0*
Glucose (mg/dl)[b]	95 ± 6	102 ± 6
Total Protein (g/dl)[b]	6.9 ± 0.1	7.0 ± 0.1
Albumin (g/dl)[g]	5.1 ± 0.1	5.1 ± 0.1
Alanine Aminotransferase (U/l)[b]	34 ± 3	30 ± 3
Aspartate Aminotransferase (U/l)[b]	83 ± 14	78 ± 11
Calcium (mg/dl)[b]	10.7 ± 0.2	10.5 ± 0.2
Inorganic Phosphorus (mg/dl)[b]	7.2 ± 0.3	8.3 ± 0.3*

[a] Values are given as LS means ± standard error of the mean. Significance at P≤0.05 is indicated by "*".
[b] n=12/group
[c] n=3 controls 4 dose
[d] n=10 controls 9 dose
[e] n=10 controls 11 dose
[f] n=9 controls 11 dose
[g] n=11 controls 11 dose

TABLE E7
Urinalysis Data for Mice in the 14-Day Drinking Water Study of Aloe vera Extracts[a]

	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
Gel Extract						
Week 1						
Male						
Volume (ml/24h)[b]	0.7 ± 0.3	1.3 ± 0.3	1.0 ± 0.3	1.9 ± 0.3	1.7 ± 0.3	1.2 ± 0.3
Urine Creatinine (mg/dl)[b]	79.8 ± 6.4	74.0 ± 6.4	74.8 ± 6.4	77.8 ± 6.4	76.0 ± 6.4	76.3 ± 6.4
Micro Protein (mg/dl)[c]	100.0 ± 2.3	97.8 ± 2.3	99.3 ± 2.3	89.6 ± 2.3*	97.1 ± 2.3	96.2 ± 3.2
Urine Glucose (mg/dl)[c]	128.0 ± 16.4	121.5 ± 16.4	108.5 ± 16.4	103.3 ± 16.4	98.0 ± 16.4	66.0 ± 23.2
Urine Creatinine (mg/24h)[b]	0.6 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	1.4 ± 0.2*	1.2 ± 0.2	0.8 ± 0.2
Micro Protein (mg/24h)[c]	0.7 ± 0.2	1.2 ± 0.2	1.0 ± 0.2	1.7 ± 0.2*	1.7 ± 0.2*	2.1 ± 0.3*
Urine Glucose (mg/24h)[c]	0.9 ± 0.3	1.4 ± 0.3	1.2 ± 0.3	1.9 ± 0.3	1.7 ± 0.3	1.4 ± 0.4
Female						
Volume (ml/24h)[b]	1.3 ± 0.2	1.2 ± 0.2	1.1± 0.2	1.3 ± 0.2	1.4 ± 0.2	1.7 ± 0.2
Urine Creatinine (mg/dl)[b]	88.3 ± 8.6	62.5 ± 8.6	86.0 ± 8.6	82.0 ± 8.6	74.3 ± 8.6	66.5 ± 8.6
Micro Protein (mg/dl)[b]	73.2 ± 5.7	66.0 ± 5.7	87.4 ± 5.7	67.1 ± 5.7	68.9 ± 5.7	75.0 ± 5.7
Urine Glucose (mg/dl)[b]	145.0 ± 34.9	118.8 ± 34.9	154.8 ± 34.9	111.5 ± 34.9	148.0 ± 34.9	105.5 ± 34.9
Urine Creatinine (mg/24h)[b]	1.1 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
Micro Protein (mg/24h)[b]	0.9 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	1.2 ± 0.2
Urine Glucose (mg/24h)[b]	1.8 ± 0.5	1.4 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	2.0 ± 0.5	1.8 ± 0.5
Week 2						
Male						
Volume (ml/24h)[b]	1.1 ± 0.3	1.4 ± 0.3	1.2 ± 0.3	1.4 ± 0.3	1.3 ± 0.3	1.2 ± 0.3
Urine Creatinine (mg/dl)[b]	84.5 ± 8.8	69.3 ± 8.8	85.5 ± 8.8	79.0 ± 8.8	77.3 ± 8.8	76.0 ± 8.8
Micro Protein (mg/dl)[b]	86.1 ± 1.8	81.4 ± 1.8	81.7 ± 1.8	86.6 ± 1.8	82.5 ± 1.8	83.0 ± 1.8
Urine Glucose (mg/dl)[b]	106.8 ± 16.6	85.0 ± 16.6	89.5 ± 16.6	83.8 ± 16.6	128.8 ± 16.6	79.5 ± 16.6
Urine Creatinine (mg/24h)[b]	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	0.9 ± 0.2
Micro Protein (mg/24h)[b]	0.9 ± 0.3	1.1 ± 0.3	0.9 ± 0.3	1.2 ± 0.3	1.0 ± 0.3	1.0 ± 0.3
Urine Glucose (mg/24h)[b]	1.0 ± 0.3	1.2 ± 0.3	0.9 ± 0.3	1.1 ± 0.3	1.6 ± 0.3	0.9 ± 0.3
Female						
Volume (ml/24h)[b]	1.4 ± 0.7	1.2 ± 0.8	3.4 ± 0.8	1.4 ± 0.7	1.6 ± 0.7	1.5 ± 0.7
Urine Creatinine (mg/dl)[b]	69.0 ± 11.5	82.7 ± 13.3	60.3 ± 13.3	72.0 ± 11.5	73.0 ± 11.5	70.0 ± 11.5
Micro Protein (mg/dl)[b]	65.6 ± 2.1	59.5 ± 2.5	60.2 ± 2.5	65.4 ± 2.1	57.2 ± 2.1	57.9 ± 2.1
Urine Glucose (mg/dl)[b]	144.3 ± 24.3	116.3 ± 28.1	90.3 ± 28.1	133.8 ± 24.3	116.5 ± 24.3	114.3 ± 24.3
Urine Creatinine (mg/24h)[b]	0.8 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
Micro Protein (mg/24h)[b]	0.9 ± 0.4	0.7 ± 0.5	2.1 ± 0.5	0.9 ± 0.4	0.9 ± 0.4	0.9 ± 0.4
Urine Glucose (mg/24h)[b]	1.9 ± 0.5	1.4 ± 0.6	1.5 ± 0.6	2.0 ± 0.5	1.8 ± 0.5	1.7 ± 0.5
Decolorized Whole Leaf Extract						
Week 1						
Male						
Volume (ml/24h)[b]	1.4 ± 0.2	1.0 ± 0.2	1.3 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	1.3 ± 0.2
Urine Creatinine (mg/dl)[b]	87.8 ± 9.2	104.3 ± 9.2	94.8 ± 9.2	102.0 ± 9.2	101.3 ± 9.2	87.8 ± 9.2
Micro Protein (mg/dl)[b]	87.9 ± 1.9	87.9 ± 1.9	90.9 ± 1.9	91.5 ± 1.9	88.8 ± 1.9	88.1 ± 1.9
Urine Glucose (mg/dl)[b]	82.3 ± 52.9	88.8 ± 52.9	129.8 ± 52.9	107.0 ± 52.9	217.3 ± 52.9	150.8 ± 52.9
Urine Creatinine (mg/24h)[b]	1.2 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	1.1 ± 0.2
Micro Protein (mg/24h)[b]	1.2 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
Urine Glucose (mg/24h)[b]	1.0 ± 0.4	0.8 ± 0.4	1.7 ± 0.4	1.0 ± 0.4	2.1 ± 0.4	1.7 ± 0.4
Female						
Volume (ml/24h)[d]	0.6 ± 0.2	0.9 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	1.0 ± 0.2
Urine Creatinine (mg/dl)[d]	117.0 ± 10.1	110.5 ± 14.2	104.0 ± 10.1	122.0 ± 10.1	93.3 ± 11.6	89.5 ± 10.1
Micro Protein (mg/dl)[e]	81.9 ± 3.2	67.4 ± 6.3	75.3 ± 3.2	80.0 ± 3.2	73.7 ± 3.7	67.5 ± 3.2*
Urine Glucose (mg/dl)[d]	310.8 ± 89.4	97.5 ± 126.4	140.3 ± 89.4	122.0 ± 89.4	159.0 ± 103.2	147.0 ± 89.4
Urine Creatinine (mg/24h)[d]	0.7 ± 0.2	0.8 ± 0.3	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.9 ± 0.2
Micro Protein (mg/24h)[e]	0.5 ± 0.1	1.1 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
Urine Glucose (mg/24h)[b]	1.3 ± 0.3	0.9 ± 0.4	0.8 ± 0.3	0.9 ± 0.3	1.3 ± 0.4	1.5 ± 0.3

TABLE E7
Urinalysis Data for Mice in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
Decolorized Whole Leaf Extract (continued)						
Week 2						
Male						
Volume (ml/24h)[b]	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	1.2 ± 0.2
Urine Creatinine (mg/dl)[b]	93.8 ± 7.8	93.5 ± 7.8	95.5 ± 7.8	91.3 ± 7.8	80.3 ± 7.8	95.0 ± 7.8
Micro Protein (mg/dl)[b]	83.8 ± 2.3	87.6 ± 2.3	88.3 ± 2.3	85.0 ± 2.3	83.1 ± 2.3	90.2 ± 2.3
Urine Glucose (mg/dl)[b]	32.8 ± 23.3	102.0 ± 23.3	171.5 ± 23.3*	32.3 ± 23.3	104.0 ± 23.3	123.8 ± 23.3
Urine Creatinine (mg/24h)[b]	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	1.1 ± 0.2
Micro Protein (mg/24h)[b]	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.1 ± 0.1
Urine Glucose (mg/24h)[b]	0.3 ± 0.3	1.1 ± 0.3	1.8 ± 0.3*	0.2 ± 0.3	0.9 ± 0.3	1.5 ± 0.3*
Female						
Volume (ml/24h)[b]	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	1.0 ± 0.2
Urine Creatinine (mg/dl)[b]	101.5 ± 9.7	98.0 ± 9.7	109.0 ± 9.7	96.8 ± 9.7	77.3 ± 9.7	82.3 ± 9.7
Micro Protein (mg/dl)[b]	83.1 ± 4.3	71.5 ± 4.3	73.5 ± 4.3	68.2 ± 4.3	71.2 ± 4.3	79.9 ± 4.3
Urine Glucose (mg/dl)[b]	69.8 ± 65.2	154.0 ± 65.2	127.0 ± 65.2	184.0 ± 65.2	126.5 ± 65.2	314.3 ± 65.2
Urine Creatinine (mg/24h)[b]	0.5 ± 0.2	0.5 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.5 ± 0.2	0.8 ± 0.2
Micro Protein (mg/24h)[b]	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.8 ± 0.2
Urine Glucose (mg/24h)[b]	0.4 ± 0.8	0.8 ± 0.8	0.9 ± 0.8	1.9 ± 0.8*	0.9 ± 0.8	3.5 ± 0.8*
Whole Leaf Extract						
Week 1						
Male						
Volume (ml/24h)[d]	0.4 ± 0.3	0.6 ± 0.2	0.3 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.5 ± 0.2
Urine Creatinine (mg/dl)[d]	86.0 ± 13.1	104.3 ± 9.3	141.3 ± 10.7*	78.3 ± 9.3	75.8 ± 9.3	85.0 ± 9.3
Micro Protein (mg/dl)[e]	90.8 ± 4.8	95.4 ± 3.4	99.8 ± 4.8	90.2 ± 3.4	88.7 ± 3.4	83.3 ± 3.4
Urine Glucose (mg/dl)[e]	561.5 ± 291.3	564.0 ± 206.0	318.0 ± 237.8	63.3 ± 237.8	566.3 ± 206.0	339.5 ± 206.0
Urine Creatinine (mg/24h)[d]	0.3 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
Micro Protein (mg/24h)[e]	0.3 ± 0.2	0.6 ± 0.2	0.4 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.4 ± 0.2
Urine Glucose (mg/24h)[d]	1.7 ± 1.7	3.0 ± 1.2	0.9 ± 1.4	0.3 ± 1.2	3.8 ± 1.2	1.8 ± 1.2
Female						
Volume (ml/24h)[b]	0.6 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.2	0.8 ± 0.1	0.5 ± 0.1
Urine Creatinine (mg/dl)[b]	101.0 ± 11.7	77.5 ± 16.5	85.0 ± 11.7	88.0 ± 16.5	99.8 ± 11.7	112.3 ± 11.7
Micro Protein (mg/dl)[b]	79.0 ± 9.7	77.7 ± 13.8	99.7 ± 9.7	86.8 ± 13.8	74.0 ± 9.7	79.6 ± 9.7
Urine Glucose (mg/dl)[b]	120.0 ± 149.2	173.5 ± 211.0	410.3 ± 149.2	149.5 ± 211.0	94.3 ± 149.2	459.0 ± 149.2
Urine Creatinine (mg/24h)[b]	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
Micro Protein (mg/24h)[b]	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
Urine Glucose (mg/24h)[b]	0.7 ± 0.6	0.8 ± 0.9	1.6 ± 0.6	0.5 ± 0.9	0.7 ± 0.6	2.1 ± 0.6
Week 2						
Male						
Volume (ml/24h)[b]	1.8 ± 0.5	1.6 ± 0.4	0.8 ± 0.4	1.5 ± 0.4	1.7 ± 0.4	1.3 ± 0.4
Urine Creatinine (mg/dl)[b]	64.7 ± 12.7	61.3 ± 11.0	93.5 ± 11.0	92.8 ± 11.0	101.5 ± 11.0	89.8 ± 11.0
Micro Protein (mg/dl)[b]	88.7 ± 4.0	82.1 ± 3.5	89.2 ± 3.5	82.8 ± 3.5	85.8 ± 3.5	79.2 ± 3.5
Urine Glucose (mg/dl)[b]	101.0 ± 24.1	43.5 ± 20.9	31.8 ± 20.9	91.8 ± 20.9	87.5 ± 20.9	36.5 ± 20.9
Urine Creatinine (mg/24h)[b]	1.1 ± 0.4	0.8 ± 0.3	0.7 ± 0.3	1.3 ± 0.3	1.6 ± 0.3	1.1 ± 0.3
Micro Protein (mg/24h)[b]	1.6 ± 0.4	1.3 ± 0.3	0.7 ± 0.3	1.2 ± 0.3	1.4 ± 0.3	1.1 ± 0.3
Urine Glucose (mg/24h)[b]	1.7 ± 0.7	0.7 ± 0.6	0.2 ± 0.6	1.4 ± 0.6	1.9 ± 0.6	0.5 ± 0.6
Female						
Volume (ml/24h)[b]	0.4 ± 0.2	1.0 ± 0.2	0.5 ± 0.2	0.9 ± 0.2	1.3 ± 0.2	1.2 ± 0.2
Urine Creatinine (mg/dl)[b]	85.5 ± 8.5	82.8 ± 6.0	92.0 ± 8.5	89.0 ± 8.5	86.5 ± 6.0	71.5 ± 6.0
Micro Protein (mg/dl)[b]	78.0 ± 5.5	75.4 ± 3.9	77.9 ± 5.5	88.4 ± 5.5	69.4 ± 3.9	78.8 ± 3.9
Urine Glucose (mg/dl)[b]	79.0 ± 20.8	38.8 ± 14.7	47.5 ± 20.8	90.5 ± 20.8	67.8 ± 14.7	44.3 ± 14.7
Urine Creatinine (mg/24h)[b]	0.3 ± 0.2	0.8 ± 0.2	0.5 ± 0.2	0.8 ± 0.2	1.1 ± 0.2	0.8 ± 0.2
Micro Protein (mg/24h)[b]	0.3 ± 0.2	0.7 ± 0.1	0.4 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	0.9 ± 0.1
Urine Glucose (mg/24h)[b]	0.4 ± 0.2	0.4 ± 0.2	0.2 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.5 ± 0.2

TABLE E7
Urinalysis Data for Mice in the 14-Day Drinking Water Study of Aloe vera Extracts (continued)

[a] Values for the parameters tested are given as LS means ± standard error of the mean (n=4/group). Significance at P≤0.05 is indicated by "*"; under the 0% group represents the test for linear trend, under the dosed groups represents comparison to the control group based on Dunnett's test.
[b] n=24 analyzed in total
[c] n=22 analyzed in total
[d] n=21 analyzed in total
[e] n=20 analyzed in total

TABLE E8
Urinalysis Data for Mice in the 13-Week Drinking Water Study on Aloe vera Whole Leaf Extract[a]

	0%	3%
Week 4		
Male		
Total Volume (ml/24 h)[b]	1.6 ± 0.2	1.0 ± 0.2*
Urine Creatinine (mg/dl)[b]	84.3 ± 4.0	94.6 ± 4.2
Micro Protein (mg/dl)[c]	62.1 ± 4.9	77.1 ± 4.9*
Urine Glucose (mg/dl)[b]	136.0 ± 3.1	143.3 ± 3.3
Urine Creatinine (mg/24h)[b]	1.3 ± 0.1	0.9 ± 0.2
Micro Protein (mg/24h)[b]	1.0 ± 0.1	0.7 ± 0.1
Urine Glucose (mg/24h[b]	2.1 ± 0.2	1.4 ± 0.2*
Female		
Total Volume (ml/24 h)[e]	0.8 ± 0.2	0.7 ± 0.2
Urine Creatinine (mg/dl))[f]	86.1 ± 10.6	113.0 ± 9.0
Micro Protein (mg/dl)[d]	246.4 ± 25.6	184.0 ± 24.4
Urine Glucose (mg/dl)[e]	74.8 ± 4.5	85.3 ± 4.1
Urine Creatinine (mg/24h)[f]	0.7 ± 0.2	0.7 ± 0.1
Micro Protein (mg/24h)[e]	2.1 ± 0.6	1.5 ± 0.5
Urine Glucose (mg/24h[e]	0.6 ± 0.1	0.6 ± 0.1
Week 8		
Male		
Total Volume (ml/24 h)[c]	1.5 ± 0.2	1.5 ± 0.2
Urine Creatinine (mg/dl)[c]	72.3 ± 3.3	98.3 ± 3.2*
Micro Protein (mg/dl)[c]	71.5 ± 12.6	79.5 ± 12.1
Urine Glucose (mg/dl)[c]	131.3 ± 2.7	157.7 ± 2.6*
Urine Creatinine (mg/24h)[c]	1.1 ± 0.1	1.5 ± 0.1*
Micro Protein (mg/24h)[c]	0.9 ± 0.1	1.1 ± 0.1
Urine Glucose (mg/24h)[c]	2.0 ± 0.2	2.4 ± 0.2
Female		
Total Volume (ml/24 h)[h]	2.0 ± 0.3	0.8 ± 0.3*
Urine Creatinine (mg/dl)[h]	57.7 ± 19.0	149.6 ± 17.6*
Micro Protein (mg/dl)[h]	417.7 ± 104.6	345.6 ± 96.9
Urine Glucose (mg/dl)[h]	55.5 ± 8.2	113.4 ± 7.6*
Urine Creatinine (mg/24h)[g]	1.0 ± 0.2	0.9 ± 0.2
Micro Protein (mg/24h)[g]	9.4 ± 2.2	2.1 ± 1.9*
Urine Glucose (mg/24h[g]	1.0 ± 0.2	0.7 ± 0.2
Week 12		
Male		
Total Volume (ml/24 h)[b]	2.1 ± 0.3	1.9 ± 0.3
Urine Creatinine (mg/dl)[b]	64.8 ± 4.2	89.2 ± 4.2*
Micro Protein (mg/dl)[b]	37.9 ± 5.9	61.3 ± 5.9*
Urine Glucose (mg/dl)[b]	73.2 ± 0.8	80.0 ± 0.8*
Urine Creatinine (mg/24h)[b]	1.3 ± 0.2	1.7 ± 0.2
Micro Protein (mg/24h)[b]	0.8 ± 0.4	1.4 ± 0.4
Urine Glucose (mg/24h[b]	1.5 ± 0.2	1.5 ± 0.2
Female		
Total Volume (ml/24 h)[e]	1.7 ± 0.3	0.9 ± 0.3
Urine Creatinine (mg/dl))[e]	87.7 ± 16.3	146.1 ± 20.0*
Micro Protein (mg/dl)[e]	378.3 ± 82.9	383.1 ± 101.5
Urine Glucose (mg/dl)[e]	57.3 ± 3.3	67.2 ± 4.0
Urine Creatinine (mg/24h)[e]	1.1 ± 0.1	0.9 ± 0.1
Micro Protein (mg/24h)[e]	5.2 ± 0.7	1.9 ± 0.8*
Urine Glucose (mg/24h[e]	0.9 ± 0.1	0.5 ± 0.2

TABLE E8
Urinalysis Data for Mice in the 13-Week Drinking Water Study on Aloe vera Whole Leaf Extract (continued)

[a] Values are given as LS means ± standard error of the mean (n=4/group). Significance at P≤0.05 is indicated by *; for dosed groups represents comparison to the control group based on Dunnett's test.
[b] n=24 analyzed in total
[c] n=23 analyzed in total
[d] n=21 analyzed in total
[e] n=20 analyzed in total
[f] n=19 analyzed in total
[g] n=14 analyzed in total
[h] n=13 analyzed in total

APPENDIX F
ORGAN WEIGHTS AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Drinking Water Studies of Aloe vera Extracts[a]

	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
Gel Extract						
Male						
Necropsy Body Weight	174.48 ± 5.69	174.40 ± 5.69	170.59 ± 5.69	174.91 ± 5.69	176.00 ± 5.69	173.29 ± 5.69
Liver						
Absolute	5.76 ± 0.29	5.69 ± 0.29	6.26 ± 0.29	5.64 ± 0.29	6.22 ± 0.29	5.68 ± 0.29
Relative	3.31 ± 0.10	3.26 ± 0.10	3.65 ± 0.10	3.21 ± 0.10	3.53 ± 0.10	3.27 ± 0.10
Heart						
Absolute	0.71 ± 0.02	0.71 ± 0.02	0.70 ± 0.02	0.69 ± 0.02	0.73 ± 0.02	0.72 ± 0.02
Relative	0.41 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.39 ± 0.01	0.42 ± 0.01	0.41 ± 0.01
Spleen						
Absolute	0.43 ± 0.02	0.45 ± 0.02	0.46 ± 0.02	0.44 ± 0.02	0.46 ± 0.02	0.44 ± 0.02
Relative	0.25 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.26 ± 0.01
Lung						
Absolute	0.99 ± 0.07	0.98 ± 0.07	1.01 ± 0.07	1.14 ± 0.07	1.08 ± 0.07	0.96 ± 0.07
Relative	0.57 ± 0.04	0.56 ± 0.04	0.60 ± 0.04	0.65 ± 0.04	0.61 ± 0.04	0.56 ± 0.04
Thymus						
Absolute	0.35 ± 0.02	0.36 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.38 ± 0.02
Relative	0.20 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.01
Testis						
Absolute	1.12 ± 0.03	1.15 ± 0.03	1.09 ± 0.03	1.14 ± 0.03	1.18 ± 0.03	1.16 ± 0.03
Relative	0.64 ± 0.01	0.66 ± 0.01	0.64 ± 0.01	0.65 ± 0.01	0.67 ± 0.01	0.67 ± 0.01
Kidney						
Absolute	0.67 ± 0.03	0.67 ± 0.03	0.71 ± 0.03	0.69 ± 0.03	0.74 ± 0.03	0.72 ± 0.03
Relative	0.38 ± 0.01*	0.39 ± 0.01	0.41 ± 0.01*	0.40 ± 0.01*	0.42 ± 0.01*	0.41 ± 0.01*
Female						
Necropsy Body Weight	118.44 ± 4.27	127.85 ± 4.27	130.03 ± 4.27	124.40 ± 4.27	128.35 ± 4.27	129.60 ± 4.27
Liver						
Absolute	4.05 ± 0.17	4.22 ± 0.17	4.42 ± 0.17	4.01 ± 0.17	4.20 ± 0.17	4.05 ± 0.17
Relative	3.42 ± 0.10	3.30 ± 0.10	3.40 ± 0.10	3.25 ± 0.10	3.27 ± 0.10	3.12 ± 0.10
Heart						
Absolute	0.51 ± 0.02	0.54 ± 0.02	0.57 ± 0.02	0.55 ± 0.02	0.56 ± 0.02	0.54 ± 0.02
Relative	0.43 ± 0.01	0.42 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.42 ± 0.01
Spleen						
Absolute	0.33 ± 0.01	0.35 ± 0.01	0.36 ± 0.01	0.34 ± 0.01	0.36 ± 0.01	0.36 ± 0.01
Relative	0.28 ± 0.01	0.28 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.27 ± 0.01
Lung						
Absolute	0.76 ± 0.03	0.82 ± 0.03	0.84 ± 0.03	0.75 ± 0.03	0.79 ± 0.03	0.78 ± 0.03
Relative	0.65 ± 0.02	0.65 ± 0.02	0.65 ± 0.02	0.61 ± 0.02	0.62 ± 0.02	0.60 ± 0.02
Thymus						
Absolute	0.30 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.01
Relative	0.26 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.25 ± 0.01
Kidney						
Absolute	0.53 ± 0.02	0.54 ± 0.02	0.57 ± 0.02	0.55 ± 0.02	0.56 ± 0.02	0.57 ± 0.02
Relative	0.45 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	0.44 ± 0.01
Decolorized Whole Leaf Extract						
Male						
Necropsy Body Weight	176.84 ± 7.23	176.43 ± 7.23	171.01 ± 7.23	173.60 ± 7.23	178.26 ± 7.23	174.54 ± 7.23
Liver						
Absolute	6.32 ± 0.30	6.62 ± 0.30	6.43 ± 0.30	6.38 ± 0.30	6.12 ± 0.30	5.91 ± 0.30
Relative	3.57 ± 0.09*	3.74 ± 0.09	3.76 ± 0.09	3.67 ± 0.09	3.45 ± 0.09	3.39 ± 0.09

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Drinking Water Studies of Aloe vera Extracts (continued)

	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
Male (continued)						
Heart						
Absolute	0.73 ± 0.03	0.72 ± 0.03	0.70 ± 0.03	0.73 ± 0.03	0.76 ± 0.03	0.72 ± 0.03
Relative	0.41 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.43 ± 0.01	0.42 ± 0.01
Spleen						
Absolute	0.48 ± 0.02	0.45 ± 0.02	0.43 ± 0.02	0.43 ± 0.02	0.45 ± 0.02	0.44 ± 0.02
Relative	0.27 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.25 ± 0.01
Lung						
Absolute	1.04 ± 0.05	1.00 ± 0.05	0.97 ± 0.05	1.01 ± 0.05	0.97 ± 0.05	0.89 ± 0.05
Relative	0.59 ± 0.02*	0.56 ± 0.02	0.57 ± 0.02	0.59 ± 0.02	0.55 ± 0.02	0.52 ± 0.02
Thymus						
Absolute	0.37 ± 0.01	0.36 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.34 ± 0.01	0.35 ± 0.01
Relative	0.21 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.21 ± 0.01
Testis						
Absolute	1.17 ± 0.05	1.17 ± 0.05	1.13 ± 0.05	1.17 ± 0.05	1.13 ± 0.05	1.18 ± 0.05
Relative	0.66 ± 0.01	0.66 ± 0.01	0.66 ± 0.01	0.68 ± 0.01	0.63 ± 0.01	0.67 ± 0.01
Kidney						
Absolute	0.73 ± 0.03	0.73 ± 0.03	0.71 ± 0.03	0.72 ± 0.03	0.73 ± 0.03	0.71 ± 0.03
Relative	0.41 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.41 ± 0.01
Female						
Necropsy Body Weight	124.50 ± 3.60	126.66 ± 3.60	127.59 ± 3.60	128.91 ± 3.60	127.30 ± 3.60	127.30 ± 3.60
Liver						
Absolute	4.15 ± 0.11	4.39 ± 0.11	4.15 ± 0.11	4.06 ± 0.11	4.05 ± 0.11	4.13 ± 0.11
Relative	3.36 ± 0.08	3.47 ± 0.08	3.25 ± 0.08	3.16 ± 0.08	3.18 ± 0.08	3.26 ± 0.08
Heart						
Absolute	0.53 ± 0.01	0.55 ± 0.01	0.54 ± 0.01	0.54 ± 0.01	0.53 ± 0.01	0.56 ± 0.01
Relative	0.43 ± 0.01	0.44 ± 0.01	0.42 ± 0.01	0.42 ± 0.01	0.41 ± 0.01	0.44 ± 0.01
Spleen						
Absolute	0.34 ± 0.01	0.36 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.34 ± 0.01	0.34 ± 0.01
Relative	0.27 ± 0.01	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01
Lung						
Absolute	0.99 ± 0.11	0.75 ± 0.11	0.75 ± 0.11	0.74 ± 0.11	0.71 ± 0.11	0.72 ± 0.11
Relative	0.80 ± 0.09	0.59 ± 0.09	0.58 ± 0.09	0.57 ± 0.09	0.56 ± 0.09	0.57 ± 0.09
Thymus						
Absolute	0.29 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.30 ± 0.01
Relative	0.24 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.24 ± 0.01
Kidney						
Absolute	0.55 ± 0.02	0.56 ± 0.02	0.55 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	0.57 ± 0.02
Relative	0.44 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.45 ± 0.01
Whole Leaf Extract						
Male						
Necropsy Body Weight	174.41 ± 7.50*	171.69 ± 7.50	167.64 ± 7.50	163.59 ± 7.50	157.04 ± 7.50	137.55 ± 7.50*
Liver						
Absolute	5.70 ± 0.26*	5.76 ± 0.26	5.60 ± 0.26	5.67 ± 0.26	5.54 ± 0.26	4.80 ± 0.26
Relative	3.28 ± 0.10	3.37 ± 0.10	3.34 ± 0.10	3.48 ± 0.10	3.53 ± 0.10	3.47 ± 0.10
Heart						
Absolute	0.69 ± 0.03*	0.72 ± 0.03	0.69 ± 0.03	0.68 ± 0.03	0.67 ± 0.03	0.59 ± 0.03*
Relative	0.40 ± 0.01	0.42 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.43 ± 0.01	0.43 ± 0.01
Spleen						
Absolute	0.43 ± 0.02*	0.44 ± 0.02	0.48 ± 0.02	0.45 ± 0.02	0.41 ± 0.02	0.34 ± 0.02
Relative	0.25 ± 0.01	0.26 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.26 ± 0.01	0.25 ± 0.01

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Drinking Water Studies of Aloe vera Extracts (continued)

	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
Male (continued)						
Lung						
Absolute	1.19 ± 0.13	0.89 ± 0.13	0.93 ± 0.13	0.98 ± 0.13	0.90 ± 0.13	0.85 ± 0.13
Relative	0.71 ± 0.09	0.52 ± 0.09	0.55 ± 0.09	0.60 ± 0.09	0.57 ± 0.09	0.62 ± 0.09
Thymus						
Absolute	0.37 ± 0.02*	0.35 ± 0.02	0.37 ± 0.02	0.35 ± 0.02	0.35 ± 0.02	0.27 ± 0.02*
Relative	0.22 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.20 ± 0.01
Testis						
Absolute	1.18 ± 0.04	1.12 ± 0.04	1.13 ± 0.04	1.11 ± 0.04	1.14 ± 0.04	1.08 ± 0.04
Relative	0.20 ± 0.01*	0.65 ± 0.03	0.68 ± 0.03	0.68 ± 0.03	0.74 ± 0.03	0.80 ± 0.03*
Kidney						
Absolute	0.68 ± 0.02*	0.72 ± 0.02	0.69 ± 0.02	0.70 ± 0.02	0.68 ± 0.02	0.60 ± 0.02
Relative	0.39 ± 0.01*	0.42 ± 0.01	0.42 ± 0.01	0.43 ± 0.01	0.44 ± 0.01*	0.45 ± 0.01*
Female						
Necropsy Body Weight	120.18 ± 4.45*	121.73 ± 4.45	122.64 ± 4.45	116.50 ± 4.45	108.95 ± 4.45	97.89 ± 4.45*
Liver						
Absolute	3.85 ± 0.16*	4.10 ± 0.16	3.95 ± 0.16	3.68 ± 0.16	3.62 ± 0.16	3.10 ± 0.16*
Relative	3.21 ± 0.07	3.37 ± 0.07	3.23 ± 0.07	3.15 ± 0.07	3.31 ± 0.07	3.16 ± 0.07
Heart						
Absolute	0.52 ± 0.02*	0.54 ± 0.02	0.53 ± 0.02	0.53 ± 0.02	0.49 ± 0.02	0.44 ± 0.02
Relative	0.43 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	0.45 ± 0.01	0.45 ± 0.01	0.46 ± 0.01
Spleen						
Absolute	0.34 ± 0.02*	0.35 ± 0.02	0.36 ± 0.02	0.35 ± 0.02	0.30 ± 0.02	0.26 ± 0.02*
Relative	0.29 ± 0.01*	0.29 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0.27 ± 0.01	0.26 ± 0.01
Lung						
Absolute	0.74 ± 0.03	0.75 ± 0.03	0.75 ± 0.03	0.70 ± 0.03	0.73 ± 0.03	0.69 ± 0.03
Relative	0.62 ± 0.02*	0.62 ± 0.02	0.61 ± 0.02	0.60 ± 0.02	0.67 ± 0.02	0.72 ± 0.02*
Thymus						
Absolute	0.32 ± 0.02*	0.32 ± 0.02	0.34 ± 0.02	0.29 ± 0.02	0.24 ± 0.02*	0.20 ± 0.02*
Relative	0.27 ± 0.02*	0.27 ± 0.02	0.28 ± 0.02	0.25 ± 0.02	0.21 ± 0.02	0.19 ± 0.02*
Kidney						
Absolute	0.51 ± 0.02*	0.56 ± 0.02	0.58 ± 0.02*	0.55 ± 0.02	0.54 ± 0.02	0.50 ± 0.02
Relative	0.43 ± 0.01*	0.46 ± 0.01	0.47 ± 0.01	0.47 ± 0.01	0.50 ± 0.01*	0.52 ± 0.01*

[a] Significant at P≤0.05 (*); under the 0% group represents the test for linear trend, under the dosed groups represents comparison to the control group based on Dunnett's test. Values are given as LS means ± standard error of the mean (n=4/group).

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Study of Aloe vera Whole Leaf Extract[a]

	0%	1%	2%	3%
Male (subchronic)				
Necropsy body weight	324.33 ± 15.89*	292.34 ± 16.60	230.37 ± 15.89*	177.25 ± 16.60*
Brain				
Absolute	2.00 ± 0.03*	1.99 ± 0.03	1.96 ± 0.03	1.81 ± 0.03*
Relative	0.62 ± 0.12*	0.68 ± 0.12	0.96 ± 0.12	1.31 ± 0.12*
Liver				
Absolute	11.03 ± 0.63*	10.73 ± 0.66	8.39 ± 0.63*	6.26 ± 0.66*
Relative	3.40 ± 0.09	3.67 ± 0.09	3.61 ± 0.09	3.47 ± 0.09
Heart				
Absolute	1.03 ± 0.06*	0.98 ± 0.06	0.82 ± 0.06	0.69 ± 0.06*
Relative	0.32 ± 0.01*	0.33 ± 0.01	0.36 ± 0.01*	0.41 ± 0.01*
Spleen				
Absolute	0.70 ± 0.04*	0.71 ± 0.05	0.59 ± 0.04	0.44 ± 0.05*
Relative	0.22 ± 0.01*	0.24 ± 0.01*	0.25 ± 0.01*	0.24 ± 0.01*
Lung				
Absolute	1.34 ± 0.06	1.22 ± 0.06	1.17 ± 0.06	0.92 ± 0.06
Relative	0.41 ± 0.03*	0.42 ± 0.03	0.53 ± 0.03*	0.59 ± 0.03*
Thymus				
Absolute	0.27 ± 0.02*	0.20 ± 0.02*	0.19 ± 0.02*	0.13 ± 0.02*
Relative	0.08 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
Kidney				
Absolute	1.09 ± 0.05	1.12 ± 0.05	0.98 ± 0.05	0.78 ± 0.05
Relative	0.34 ± 0.02*	0.38 ± 0.02	0.45 ± 0.02*	0.48 ± 0.02*
Testes				
Absolute	1.47 ± 0.07	1.44 ± 0.07	1.32 ± 0.07	1.11 ± 0.07
Relative	0.45 ± 0.03*	0.50 ± 0.03	0.61 ± 0.03*	0.69 ± 0.03*
Female (subchronic)				
Necropsy body weight	179.42 ± 8.64*	173.36 ± 8.64	117.09 ± 8.64*	89.76 ± 9.47*
Brain				
Absolute	1.82 ± 0.03*	1.81 ± 0.03	1.70 ± 0.03*	1.64 ± 0.04*
Relative	1.02 ± 0.14*	1.05 ± 0.14	1.66 ± 0.14*	2.15 ± 0.16*
Liver				
Absolute	5.67 ± 0.34*	5.85 ± 0.34	4.51 ± 0.34	2.97 ± 0.38*
Relative	3.17 ± 0.12	3.38 ± 0.12	3.79 ± 0.12*	3.33 ± 0.13
Heart				
Absolute	0.65 ± 0.03*	0.67 ± 0.03	0.52 ± 0.03*	0.40 ± 0.03*
Relative	0.36 ± 0.02*	0.38 ± 0.02	0.45 ± 0.02*	0.47 ± 0.02*
Spleen				
Absolute	0.41 ± 0.03*	0.47 ± 0.03	0.31 ± 0.03	0.22 ± 0.03*
Relative	0.23 ± 0.01	0.27 ± 0.01*	0.26 ± 0.01	0.23 ± 0.01
Lung				
Absolute	0.96 ± 0.04*	0.91 ± 0.04	0.74 ± 0.04*	0.66 ± 0.04*
Relative	0.53 ± 0.05*	0.53 ± 0.05	0.67 ± 0.05	0.86 ± 0.05*
Thymus				
Absolute	0.20 ± 0.01*	0.18 ± 0.01	0.10 ± 0.01*	0.07 ± 0.01*
Relative	0.11 ± 0.01*	0.10 ± 0.01	0.08 ± 0.01*	0.08 ± 0.01*
Kidney				
Absolute	0.63 ± 0.03*	0.72 ± 0.03	0.61 ± 0.03	0.49 ± 0.03*
Relative	0.35 ± 0.02	0.42 ± 0.02	0.55 ± 0.02*	0.60 ± 0.02*

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Male (metabolism)				
Necropsy body weight	301.50 ± 6.38	ND[b]	216.13 ± 6.38*	ND
Brain				
Absolute	2.00 ± 0.05	ND	1.87 ± 0.05	ND
Relative	0.66 ± 0.04	ND	0.88 ± 0.04	ND
Liver				
Absolute	8.06 ± 0.24	ND	6.13 ± 0.24*	ND
Relative	2.67 ± 0.07	ND	2.85 ± 0.07	ND
Heart				
Absolute	0.96 ± 0.03	ND	0.79 ± 0.03*	ND
Relative	0.32 ± 0.01	ND	0.36 ± 0.01*	ND
Spleen				
Absolute	0.61 ± 0.02	ND	0.54 ± 0.02	ND
Relative	0.20 ± 0.00	ND	0.25 ± 0.00*	ND
Lung				
Absolute	1.22 ± 0.04	ND	1.04 ± 0.04*	ND
Relative	0.41 ± 0.02	ND	0.48 ± 0.02*	ND
Thymus				
Absolute	0.21 ± 0.01	ND	0.11 ± 0.01	ND
Relative	0.07 ± 0.00	ND	0.05 ± 0.00*	ND
Kidney				
Absolute	0.99 ± 0.03	ND	0.88 ± 0.03*	ND
Relative	0.33 ± 0.01	ND	0.41 ± 0.01*	ND
Testes				
Absolute	1.46 ± 0.05	ND	1.27 ± 0.05	ND
Relative	0.48 ± 0.02	ND	0.59 ± 0.02	ND
Female (metabolism)				
Necropsy body weight	179.48 ± 7.53	ND	133.52 ± 7.53*	ND
Brain				
Absolute	1.83 ± 0.02	ND	1.74 ± 0.02*	ND
Relative	1.02 ± 0.09	ND	1.40 ± 0.09*	ND
Liver				
Absolute	4.21 ± 0.18	ND	3.86 ± 0.18	ND
Relative	2.34 ± 0.09	ND	2.96 ± 0.09*	ND
Heart				
Absolute	0.64 ± 0.03	ND	0.53 ± 0.03*	ND
Relative	0.35 ± 0.01	ND	0.41 ± 0.01*	ND
Spleen				
Absolute	0.40 ± 0.03	ND	0.36 ± 0.03	ND
Relative	0.22 ± 0.01	ND	0.27 ± 0.01*	ND
Lung				
Absolute	0.88 ± 0.03	ND	0.75 ± 0.03*	ND
Relative	0.49 ± 0.02	ND	0.58 ± 0.02*	ND
Thymus				
Absolute	0.19 ± 0.01	ND	0.11 ± 0.01*	ND
Relative	0.11 ± 0.01	ND	0.08 ± 0.01*	ND
Kidney				
Absolute	0.59 ± 0.01	ND	0.60 ± 0.01*	ND
Relative	0.11 ± 0.01	ND	0.08 ± 0.01*	ND

[a] Significant at P≤0.05 (*); under the 0% group represents the test for linear trend, under the dosed groups represents comparison to the control group based on Dunnett's test. Values are given as LS means ± standard error of the mean (n=12/group).
[b] Not done. Only control (0%) and 2% groups were used for metabolism studies.

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Drinking Water Studies of Aloe vera Extracts[a]

	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
Gel Extract						
Male						
Necropsy Body Weight	23.59 ± 0.72	22.76 ± 0.72	23.16 ± 0.72	23.18 ± 0.72	22.58 ± 0.72	22.03 ± 0.72
Liver						
Absolute	0.99 ± 0.04*	0.96 ± 0.04	1.07 ± 0.04	1.01 ± 0.04	0.92 ± 0.04	0.87 ± 0.04
Relative	4.23 ± 0.10*	4.20 ± 0.10	4.60 ± 0.10*	4.34 ± 0.10	4.05 ± 0.10	3.95 ± 0.10
Heart						
Absolute	0.14 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
Relative	0.59 ± 0.03	0.65 ± 0.03	0.57 ± 0.03	0.59 ± 0.03	0.60 ± 0.03	0.57 ± 0.03
Spleen						
Absolute	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Relative	0.28 ± 0.02	0.33 ± 0.02	0.33 ± 0.02	0.26 ± 0.02	0.29 ± 0.02	0.26 ± 0.02
Lung						
Absolute	0.17 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
Relative	0.74 ± 0.04	0.79 ± 0.04	0.75 ± 0.04	0.74 ± 0.04	0.72 ± 0.04	0.73 ± 0.04
Thymus						
Absolute	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Relative	0.15 ± 0.02*	0.18 ± 0.02	0.15 ± 0.02	0.21 ± 0.02*	0.18 ± 0.02	0.19 ± 0.02
Testis						
Absolute	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Relative	0.42 ± 0.01	0.45 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.44 ± 0.01
Kidney						
Absolute	0.19 ± 0.01*	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01*
Relative	0.81 ± 0.03	0.82 ± 0.03	0.83 ± 0.03	0.79 ± 0.03	0.82 ± 0.03	0.75 ± 0.03
Female						
Necropsy Body Weight	18.33 ± 0.36	18.31 ± 0.36	18.19 ± 0.36	17.21 ± 0.36	18.24 ± 0.36	17.74 ± 0.36
Liver						
Absolute	0.79 ± 0.02	0.79 ± 0.02	0.76 ± 0.02	0.74 ± 0.02	0.79 ± 0.02	0.74 ± 0.02
Relative	4.30 ± 0.08	4.31 ± 0.08	4.16 ± 0.08	4.29 ± 0.08	4.35 ± 0.08	4.18 ± 0.08
Heart						
Absolute	0.12 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Relative	0.65 ± 0.03	0.64 ± 0.03	0.58 ± 0.03	0.64 ± 0.03	0.64 ± 0.03	0.64 ± 0.03
Spleen						
Absolute	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.06 ± 0.00
Relative	0.36 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	0.35 ± 0.01	0.36 ± 0.01	0.36 ± 0.01
Lung						
Absolute	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01
Relative	0.84 ± 0.05	0.84 ± 0.05	0.89 ± 0.05	0.92 ± 0.05	0.96 ± 0.05	0.86 ± 0.05
Thymus						
Absolute	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Relative	0.28 ± 0.02	0.28 ± 0.02	0.30 ± 0.02	0.27 ± 0.02	0.29 ± 0.02	0.28 ± 0.02
Kidney						
Absolute	0.14 ± 0.00	0.14 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00
Relative	0.77 ± 0.02	0.74 ± 0.02	0.72 ± 0.02	0.78 ± 0.02	0.74 ± 0.02	0.72 ± 0.02
Decolorized Whole Leaf Extract						
Male						
Necropsy Body Weight	22.38 ± 0.57	24.20 ± 0.57	22.00 ± 0.57	23.84 ± 0.57	22.73 ± 0.57	24.14 ± 0.57
Liver						
Absolute	1.00 ± 0.04	1.02 ± 0.04	0.90 ± 0.04	1.09 ± 0.04	0.95 ± 0.04	1.01 ± 0.04
Relative	4.45 ± 0.10	4.21 ± 0.10	4.10 ± 0.10	4.57 ± 0.10	4.17 ± 0.10	4.19 ± 0.10

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Drinking Water Studies of Aloe vera Extracts (continued)

	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
Male (continued)						
Heart						
Absolute	0.12 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01
Relative	0.56 ± 0.02	0.56 ± 0.02	0.59 ± 0.02	0.55 ± 0.02	0.59 ± 0.02	0.58 ± 0.02
Spleen						
Absolute	0.11 ± 0.01	0.07 ± 0.01	0.06 ± 0.01*	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
Relative	0.51 ± 0.07	0.29 ± 0.07	0.27 ± 0.07	0.29 ± 0.07	0.27 ± 0.07	0.28 ± 0.07
Lung						
Absolute	0.16 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01
Relative	0.73 ± 0.03	0.73 ± 0.03	0.78 ± 0.03	0.71 ± 0.03	0.74 ± 0.03	0.74 ± 0.03
Thymus						
Absolute	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Relative	0.16 ± 0.02	0.17 ± 0.02	0.16 ± 0.02	0.17 ± 0.02	0.19 ± 0.02	0.18 ± 0.02
Testis						
Absolute	0.09 ± 0.00	0.10 ± 0.00	0.09 ± 0.00	0.10 ± 0.00	0.09 ± 0.00	0.10 ± 0.00
Relative	0.42 ± 0.01	0.41 ± 0.01	0.43 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.41 ± 0.01
Kidney						
Absolute	0.18 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.01
Relative	0.81 ± 0.02	0.79 ± 0.02	0.78 ± 0.02	0.79 ± 0.02	0.78 ± 0.02	0.77 ± 0.02
Female						
Necropsy Body Weight	17.99 ± 0.54	18.10 ± 0.54	17.98 ± 0.54	17.35 ± 0.54	17.65 ± 0.54	17.05 ± 0.54
Liver						
Absolute	0.80 ± 0.03	0.72 ± 0.03	0.73 ± 0.03	0.72 ± 0.03	0.73 ± 0.03	0.73 ± 0.03
Relative	4.45 ± 0.12	4.04 ± 0.12	4.05 ± 0.12	4.16 ± 0.12	4.14 ± 0.12	4.27 ± 0.12
Heart						
Absolute	0.11 ± 0.00	0.10 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00
Relative	0.63 ± 0.03	0.57 ± 0.03	0.58 ± 0.03	0.64 ± 0.03	0.60 ± 0.03	0.64 ± 0.03
Spleen						
Absolute	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
Relative	0.36 ± 0.02	0.33 ± 0.02	0.35 ± 0.02	0.34 ± 0.02	0.33 ± 0.02	0.34 ± 0.02
Lung						
Absolute	0.16 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
Relative	0.87 ± 0.05	0.83 ± 0.05	0.90 ± 0.05	0.89 ± 0.05	0.86 ± 0.05	0.89 ± 0.05
Thymus						
Absolute	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.00
Relative	0.31 ± 0.02	0.28 ± 0.02	0.34 ± 0.02	0.32 ± 0.02	0.32 ± 0.02	0.32 ± 0.02
Kidney						
Absolute	0.13 ± 0.00	0.12 ± 0.00	0.13 ± 0.00	0.12 ± 0.00	0.13 ± 0.00	0.13 ± 0.00
Relative	0.75 ± 0.02	0.69 ± 0.02	0.72 ± 0.02	0.71 ± 0.02	0.73 ± 0.02	0.76 ± 0.02
Whole Leaf Extract						
Male						
Necropsy Body Weight	21.49 ± 0.61*	23.24 ± 0.61	22.95 ± 0.61	22.30 ± 0.61	23.28 ± 0.61	24.43 ± 0.61*
Liver						
Absolute	0.87 ± 0.03*	0.94 ± 0.03	0.93 ± 0.03	0.95 ± 0.03	0.95 ± 0.03	1.04 ± 0.03*
Relative	4.06 ± 0.13	4.03 ± 0.13	4.07 ± 0.13	4.25 ± 0.13	4.08 ± 0.13	4.31 ± 0.13
Heart						
Absolute	0.13 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
Relative	0.60 ± 0.03	0.62 ± 0.03	0.56 ± 0.03	0.61 ± 0.03	0.56 ± 0.03	0.56 ± 0.03
Spleen						
Absolute	0.06 ± 0.01*	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.10 ± 0.01
Relative	0.26 ± 0.06*	0.26 ± 0.06	0.27 ± 0.06	0.31 ± 0.06	0.30 ± 0.06	0.42 ± 0.06

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Drinking Water Studies of Aloe vera Extracts (continued)

	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
Male (continued)						
Lung						
Absolute	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	0.17 ± 0.01
Relative	0.80 ± 0.03	0.73 ± 0.03	0.73 ± 0.03	0.73 ± 0.03	0.76 ± 0.03	0.70 ± 0.03
Thymus						
Absolute	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Relative	0.19 ± 0.02	0.17 ± 0.02	0.17 ± 0.02	0.20 ± 0.02	0.18 ± 0.02	0.18 ± 0.02
Testis						
Absolute	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Relative	0.46 ± 0.01*	0.43 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.41 ± 0.01*	0.40 ± 0.01*
Kidney						
Absolute	0.18 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.19 ± 0.01
Relative	0.82 ± 0.03	0.81 ± 0.03	0.81 ± 0.03	0.82 ± 0.03	0.85 ± 0.03	0.79 ± 0.03
Female						
Necropsy Body Weight	18.26 ± 0.57	18.20 ± 0.57	19.03 ± 0.57	17.64 ± 0.57	18.13 ± 0.57	17.31 ± 0.57
Liver						
Absolute	0.76 ± 0.03	0.76 ± 0.03	0.76 ± 0.03	0.74 ± 0.03	0.78 ± 0.03	0.70 ± 0.03
Relative	4.19 ± 0.10	4.17 ± 0.10	4.02 ± 0.10	4.20 ± 0.10	4.28 ± 0.10	4.03 ± 0.10
Heart						
Absolute	0.13 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
Relative	0.70 ± 0.04	0.64 ± 0.04	0.58 ± 0.04	0.69 ± 0.04	0.63 ± 0.04	0.68 ± 0.04
Spleen						
Absolute	0.06 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
Relative	0.33 ± 0.02	0.37 ± 0.02	0.33 ± 0.02	0.35 ± 0.02	0.34 ± 0.02	0.34 ± 0.02
Lung						
Absolute	0.19 ± 0.01*	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.14 ± 0.01*
Relative	1.02 ± 0.04*	0.86 ± 0.04*	0.84 ± 0.04*	0.92 ± 0.04	0.95 ± 0.04	0.81 ± 0.04*
Thymus						
Absolute	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.00
Relative	0.28 ± 0.02	0.31 ± 0.02	0.27 ± 0.02	0.31 ± 0.02	0.30 ± 0.02	0.28 ± 0.02
Kidney						
Absolute	0.14 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.13 ± 0.00
Relative	0.77 ± 0.02	0.73 ± 0.02	0.71 ± 0.02	0.79 ± 0.02	0.77 ± 0.02	0.76 ± 0.02

[a] Significant at P≤0.05 (*); under the 0% group represents the test for linear trend, under the dosed groups represents comparison to the control group based on Dunnett's test. Values are given as LS means ± standard error of the mean (n=4/group).

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Study of Aloe vera Whole Leaf Extract[a]

	0%	1%	2%	3%
Male (subchronic)				
Necropsy body weight	30.08 ± 0.71	29.36 ± 0.71	29.01 ± 0.71	28.48 ± 0.71
Brain				
Absolute	0.48 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.47 ± 0.01
Relative	1.6 ± 0.05	1.65 ± 0.05	1.66 ± 0.05	1.67 ± 0.05
Liver				
Absolute	1.36 ± 0.03	1.37 ± 0.03	1.33 ± 0.03	1.33 ± 0.03
Relative	0.6 ± 0.14	0.59 ± 0.14	0.6 ± 0.14	0.63 ± 0.14
Heart				
Absolute	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01
Relative	0.6 ± 0.02	0.59 ± 0.02	0.6 ± 0.02	0.63 ± 0.02
Spleen				
Absolute	0.08 ± 0.00	0.09 ± 0.00	0.07 ± 0.00	0.08 ± 0.00
Relative	0.26 ± 0.01	0.29 ± 0.01	0.25 ± 0.01	0.27 ± 0.01
Lung				
Absolute	0.2 ± 0.01	0.2 ± 0.01	0.19 ± 0.01	0.2 ± 0.01
Relative	0.65 ± 0.03	0.66 ± 0.03	0.67 ± 0.03	0.7 ± 0.03
Thymus				
Absolute	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Relative	0.1 ± 0.01	0.11 ± 0.01	0.1 ± 0.01	0.11 ± 0.01
Kidney				
Absolute	0.25 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.24 ± 0.01
Relative	0.82 ± 0.03	0.86 ± 0.03	0.84 ± 0.03	0.85 ± 0.03
Testes				
Absolute	0.12 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.11 ± 0.00
Relative	0.39 ± 0.01	0.4 ± 0.01	0.39 ± 0.01	0.4 ± 0.01
Female (subchronic)				
Necropsy body weight	23.43 ± 0.51	23.05 ± 0.51	23.43 ± 0.53	23.17 ± 0.51
Brain				
Absolute	0.48 ± 0.01	0.49 ± 0.01	0.49 ± 0.01	0.48 ± 0.01
Relative	2.07 ± 0.04	2.11 ± 0.04	2.11 ± 0.04	2.09 ± 0.04
Liver				
Absolute	1.02 ± 0.03	1.08 ± 0.03	1.01 ± 0.03	1.01 ± 0.03
Relative	4.33 ± 0.07	4.69 ± 0.07*	4.33 ± 0.08	4.36 ± 0.07
Heart				
Absolute	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
Relative	0.64 ± 0.03	0.64 ± 0.03	0.66 ± 0.03	0.60 ± 0.03
Spleen				
Absolute	0.07 ± 0.00	0.08 ± 0.00*	0.07 ± 0.00	0.07 ± 0.00
Relative	0.30 ± 0.01	0.36 ± 0.01*	0.30 ± 0.01	0.32 ± 0.01
Lung				
Absolute	0.19 ± 0.02	0.23 ± 0.02	0.20 ± 0.02	0.18 ± 0.02
Relative	0.81 ± 0.07	0.99 ± 0.07	0.87 ± 0.07	0.79 ± 0.07
Thymus				
Absolute	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Relative	0.16 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
Kidney				
Absolute	0.17 ± 0.00	0.18 ± 0.00*	0.17 ± 0.00	0.18 ± 0.00
Relative	0.71 ± 0.01	0.79 ± 0.01*	0.72 ± 0.01	0.77 ± 0.01*

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Study of Aloe vera Whole Leaf Extract (continued)

	0%	1%	2%	3%
Male (metabolism)				
Necropsy body weight	28.75 ± 0.43	ND[b]	ND	27.97 ± 0.43
Brain				
Absolute	0.47 ± 0.01	ND	ND	0.47 ± 0.01
Relative	1.64 ± 0.03	ND	ND	1.70 ± 0.03
Liver				
Absolute	1.05 ± 0.02	ND	ND	1.05 ± 0.02
Relative	3.66 ± 0.06	ND	ND	3.76 ± 0.06
Heart				
Absolute	0.16 ± 0.01	ND	ND	0.16 ± 0.01
Relative	0.57 ± 0.02	ND	ND	0.57 ± 0.02
Spleen				
Absolute	0.07 ± 0.00	ND	ND	0.07 ± 0.00
Relative	0.24 ± 0.01	ND	ND	0.24 ± 0.01
Lung				
Absolute	0.19 ± 0.01	ND	ND	0.19 ± 0.01
Relative	0.66 ± 0.02	ND	ND	0.69 ± 0.02
Thymus				
Absolute	0.03 ± 0.00	ND	ND	0.04 ± 0.00
Relative	0.11 ± 0.01	ND	ND	0.13 ± 0.01
Kidney				
Absolute	0.22 ± 0.00	ND	ND	0.22 ± 0.00
Relative	0.75 ± 0.01	ND	ND	0.77 ± 0.01
Testes				
Absolute	0.12 ± 0.00	ND	ND	0.12 ± 0.00
Relative	0.40 ± 0.01	ND	ND	0.41 ± 0.01
Female (metabolism)				
Necropsy body weight	20.82 ± 0.18	ND	ND	21.12 ± 0.18
Brain				
Absolute	0.47 ± 0.00	ND	ND	0.48 ± 0.00
Relative	2.27 ± 0.03	ND	ND	2.27 ± 0.03
Liver				
Absolute	0.78 ± 0.01	ND	ND	0.81 ± 0.01
Relative	3.74 ± 0.04	ND	ND	3.81 ± 0.04
Heart				
Absolute	0.13 ± 0.00	ND	ND	0.13 ± 0.00
Relative	0.62 ± 0.02	ND	ND	0.62 ± 0.02
Spleen				
Absolute	0.06 ± 0.00	ND	ND	0.06 ± 0.00
Relative	0.29 ± 0.01	ND	ND	0.30 ± 0.01
Lung				
Absolute	0.18 ± 0.01	ND	ND	0.17 ± 0.01
Relative	0.86 ± 0.04	ND	ND	0.81 ± 0.04
Thymus				
Absolute	0.03 ± 0.00	ND	ND	0.04 ± 0.00
Relative	0.16 ± 0.01	ND	ND	0.17 ± 0.01
Kidney				
Absolute	0.14 ± 0.00	ND	ND	0.15 ± 0.00*
Relative	0.67 ± 0.01	ND	ND	0.71 ± 0.01*

[a] Significant at P≤0.05 (*); under the 0% group represents the test for linear trend, under the dosed groups represents comparison to the control group based on Dunnett's test. Values are given as LS means ± standard error of the mean (n=12/group).
[b] Not done. Only control (0%) and 3% groups were used for metabolism studies.

APPENDIX G
GASTROINTESTINAL TRANSIT DATA

TABLE G1
Gastrointestinal Transit Data for Rats in the 14-Day Drinking Water Study of Aloe vera Extracts[a]

	Male		Female	
	Week 1	Week 2	Week 1	Week 2
Gel Extract				
0.0%	9.00 ± 0.40	7.50 ± 0.61	9.25 ± 0.74	8.25 ± 1.22
0.5%	8.75 ± 0.40	9.25 ± 0.61	10.25 ± 0.74	8.25 ± 1.22
1.0%	8.50 ± 0.40	7.00 ± 0.61	8.00 ± 0.74	9.25 ± 1.22
1.5%	8.00 ± 0.40	7.00 ± 0.61	8.50 ± 0.74	7.75 ± 1.22
2.0%	8.25 ± 0.40	8.75 ± 0.61	9.00 ± 0.74	9.00 ± 1.22
3.0%	8.50 ± 0.40	7.00 ± 0.61	7.50 ± 0.74	8.00 ± 1.22
Decolorized Whole Leaf Extract				
0.0%	8.75 ± 0.46	6.75 ± 0.51	8.75 ± 0.74	7.25 ± 0.70
0.5%	7.50 ± 0.46	7.25 ± 0.51	9.25 ± 0.74	7.75 ± 0.70
1.0%	7.50 ± 0.46	8.25 ± 0.51	9.75 ± 0.74	8.50 ± 0.70
1.5%	8.50 ± 0.46	7.25 ± 0.51	8.25 ± 0.74	8.50 ± 0.70
2.0%	8.25 ± 0.46	7.00 ± 0.51	8.25 ± 0.74	7.75 ± 0.70
3.0%	9.25 ± 0.46	9.00 ± 0.51*	9.00 ± 0.74	8.75 ± 0.70
Whole Leaf Extract				
0.0%	10.25 ± 0.60	9.25 ± 0.67	12.75 ± 0.79	8.75 ± 0.65
0.5%	8.50 ± 0.60	6.75 ± 0.67	9.50 ± 0.79*	8.25 ± 0.65
1.0%	7.75 ± 0.60*	6.50 ± 0.67*	9.00 ± 0.79*	6.50 ± 0.65
1.5%	7.00 ± 0.60*	6.00 ± 0.67*	8.75 ± 0.79*	6.75 ± 0.65
2.0%	6.50 ± 0.60*	5.75 ± 0.67*	9.00 ± 0.79*	8.00 ± 0.65
3.0%	8.33 ± 0.69	6.50 ± 0.67*	8.75 ± 0.79*	4.75 ± 0.65*

[a] Gastrointestinal transit time is expressed in hours. Significant at P≤0.05 (*); under the 0% group represents the test for linear trend, under the dosed groups represents comparison to the control group based on Dunnett's test. Values are given as LS means ± standard error of the mean (n=4/group).

TABLE G2
Gastrointestinal Transit Data for Rats
in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract[a,b]

	Week 4	Week 8	Week 12
Male			
0.0%	9.5 ± 0.3	9.8 ± 0.6	11.5 ± 0.3
2.0%	6.6 ± 0.3*	7.2 ± 0.6*	4.3 ± 0.3*
Female			
0.0%	10.3 ± 0.7	10.2 ± 0.4	11.0 ± 0.2
2.0%	7.1 ± 0.7*	6.0 ± 0.5*	6.2 ± 0.3*

[a] Gastrointestinal transit time is expressed in hours; n=12 males /group or 12 female controls and 9 female 2%
[b] Values are given as LS mean ± standard error of the mean.
* Signifies values that are significantly (P ≤ 0.05) different from the control group by Tukey's tests.

TABLE G3
Gastrointestinal Transit Data for Mice in the 14-Day Drinking Water Study of Aloe vera Extracts[a]

	Male		Female	
	Week 1	**Week 2**	**Week 1**	**Week 2**
Gel Extract				
0.0%	4.25 ± 0.46*	5.00 ± 0.37	4.50 ± 0.53	5.00 ± 0.43
0.5%	5.25 ± 0.46	4.75 ± 0.37	5.25 ± 0.53	4.50 ± 0.43
1.0%	4.50 ± 0.46	4.00 ± 0.37	5.25 ± 0.53	4.25 ± 0.43
1.5%	3.75 ± 0.46	5.00 ± 0.37	4.25 ± 0.53	5.00 ± 0.43
2.0%	3.50 ± 0.46	4.50 ± 0.37	4.75 ± 0.53	5.25 ± 0.43
3.0%	3.75 ± 0.46	4.00 ± 0.37	5.00 ± 0.53	4.25 ± 0.43
Decolorized Whole Leaf Extract				
0.0%	6.25 ± 0.38	5.25 ± 0.37*	6.50 ± 0.49*	5.75 ± 0.58
0.5%	4.75 ± 0.38*	4.25 ± 0.37	6.25 ± 0.49	5.00 ± 0.58
1.0%	5.00 ± 0.38	4.75 ± 0.37	5.25 ± 0.49	5.00 ± 0.58
1.5%	5.00 ± 0.38	5.00 ± 0.37	5.50 ± 0.49	4.75 ± 0.58
2.0%	6.50 ± 0.38	4.75 ± 0.37	4.50 ± 0.49*	4.75 ± 0.58
3.0%	5.00 ± 0.38	3.50 ± 0.37*	5.50 ± 0.49	5.00 ± 0.58
Whole Leaf Extract				
0.0%	5.00 ± 0.34*	4.50 ± 0.22	5.25 ± 0.49	5.00 ± 0.43
0.5%	4.25 ± 0.34	5.00 ± 0.22	5.25 ± 0.49	4.50 ± 0.43
1.0%	4.25 ± 0.34	4.50 ± 0.22	4.00 ± 0.49	4.75 ± 0.43
1.5%	4.00 ± 0.34	4.00 ± 0.22	5.00 ± 0.49	3.75 ± 0.43
2.0%	4.00 ± 0.34	3.75 ± 0.22	4.50 ± 0.49	4.00 ± 0.43
3.0%	3.50 ± 0.34*	4.75 ± 0.22	4.75 ± 0.49	5.75 ± 0.43

[a] Gastrointestinal transit time is expressed in hours; n=4 mice/group.
* Signifies significant linear dose trend ($P \leq 0.05$) effects based on contrast comparisons for the control group and in treatment groups signifies mean values that are significantly different ($P \leq 0.05$) from control group by Dunnett's test.

TABLE G4
Gastrointestinal Transit Data for Mice
in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract[a,b]

	Week 4	**Week 8**	**Week 12**
Male			
0%	6.6 ± 0.2	4.5 ± 0.3	5.3 ± 0.2
3%	6.8 ± 0.2	**5.6 ± 0.3***	5.2 ± 0.2
Female			
0%	7.8 ± 0.2	6.3 ± 0.3	5.8 ± 0.2
3%	7.5 ± 0.2	6.2 ± 0.3	6.2 ± 0.2

[a] Gastrointestinal transit time is expressed in hours; n=12 mice/group.
[b] Values are given as mean ± standard error of the mean.
* Signifies values that are significantly ($P \leq 0.05$) different from the control group by Tukey's tests.

APPENDIX H
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF ALOE VERA EXTRACTS

The lyophilized (max. 6% moisture content) Aloe vera extracts used in the 14-day, 13-week, and 2-year studies were obtained from Pangea Phytoceuticals, Inc. (Harlingen, TX). For the 14-day study, extracts included *Aloe barbadensis* Process A gel (Aloe vera gel), *Aloe barbadensis* non-decolorized whole leaf (Aloe vera whole leaf), and *Aloe barbadensis* decolorized whole leaf (Aloe vera decolorized whole leaf) extracts. The 13-week and 2-year studies used only the Aloe vera whole leaf extract. The Aloe vera gel extract consisted of the inner leaf gel of hand-filleted Aloe vera leaves with the pulp removed. The Aloe vera whole leaf extract was produced by grinding the whole leaves of Aloe vera plants and treating the slurry with cellulase (23 mg/L) to reduce viscosity and maximize yields. The Aloe vera whole leaf extract contained not only the inner leaf gel but the Aloe vera latex as well. The Aloe vera decolorized whole leaf extract was prepared in an identical manner as the Aloe vera whole leaf extract, with the exception that the slurry was further treated with activated carbon (1.0% wt/wt) to remove the latex anthraquinone components from the extract.

For the 14-day studies, the Aloe vera gel extract lot numbers were 020318AG, 060308AG, 020810AG, and 022308AG; the Aloe vera whole leaf extract lot numbers were 020228ND, 060308ND, and 020928ND; and the Aloe vera decolorized whole leaf extract lot numbers were 020223AC, 060308AC, and 020916AC. For the 13-week studies, the Aloe vera whole leaf extract lot numbers were 042803ND, 032606ND, 081303ND, 082203ND, 090803ND, 093003ND, and 100203ND. For the 2-year studies, the Aloe vera whole leaf extract lot numbers were 041214ND, 040930ND, 041007ND, 041119ND, and 041210ND. Sterilization was achieved by gamma-ray irradiation. Once irradiated, the different lots of each of the Aloe vera extracts were combined and blended, and new lot numbers were assigned. For the 14-day study, Aloe vera gel extract was assigned lot numbers PA-02001 and PA-02002; Aloe vera whole leaf extract was assigned lot numbers WLN-02001 and WLN-2002; and Aloe vera decolorized whole leaf extract was assigned lot numbers WLD-02001 and WLD-2002. For the 13-week study, Aloe vera whole leaf extract lots were combined with WLN-02002, and the new lot assignment was WLN-03001. For the 2-year study, Aloe vera whole leaf extract was assigned lot numbers WLN-005001A, WLN-005001B, WLN-006001A, WLN-006001B, and WLN-006001C.

Aloe vera has high water content, ranging from 99%-99.5% (Atherton, 1998), with the remaining 0.5%-1.0% solid material reported to contain over 75 different potentially active compounds. The mucilaginous aloe gel contains a number of polysaccharides, including the major polysaccharide, acemannan. Organic acids, such as malic acid, accumulate in the aloe gel. Aloe latex is a bitter, yellow plant exudate containing a wide variety of compounds, including anthraquinone *C*-glycosides, anthrones, and free anthraquinones (Park *et al.*, 1998). Aloe latex contains four major C-glycosyl constituents: aloin A, aloin B, aloesin, and aloeresin A (Saccu *et al.*, 2001). Several other C-glycosyl-chromones and anthrones have been isolated from Aloe vera, including aloe-emodin, the anthraquinone of aloin A and aloin B (Zonta *et al.*, 1995; Okamura *et al.*, 1996; Okamura *et al.*, 1997; Saleem *et al.*, 1997; Park *et al.*, 1998). The Division of Biochemical Toxicology Chemistry Support Group at the National Center for Toxicological Research of the Jefferson Laboratories of the Food and Drug Administration was responsible for determining the homogeneity and content of malic acid and aloin A, the polysaccharide average molecular weight, and the alcohol insoluble glycosidic residues of the test articles.

The analysis for content and homogeneity was performed on nine samples from lot numbers WLN-005001A, WLN-005001B, WLN-006001A, WLN-006001B, and WLN-006001C of the 2-year carcinogenesis study. Three each were collected from the top, middle and bottom of the WLN test articles.

Samples were supplied by the Bionetics Diet Preparation staff at the National Center for Toxicological Research and were stored frozen, in a desiccator, until use. For malic acid and aloin A testing, 50 mg were weighed from each received sample into 50 ml glass beakers. All samples were extracted by the addition of approximately 15 ml 25% acetonitrile in 0.5 M sulfuric acid (25%ACN/0.5 M H_2SO_4) to each beaker, sonicated for approximately one minute and then quantitatively transferred to 50 ml volumetric flasks. Additionally, for the aloin A assay, three WLN

samples were spiked with 0.5 ml of 1 mg/ml aloin A standard. The samples were diluted to 50 ml with 25% ACN/0.5 M H_2SO_4, mixed by inversion, and then filtered through 0.45 µm nylon Pall Gelman acrodisc filters. One milliliter aliquots from each sample were then transferred into HPLC vials for subsequent analysis and comparison to the appropriate malic acid standard and aloin A standards also contained in 25%ACN/0.5 M H_2SO_4.

Malic acid analysis was performed using a Waters Millennium HPLC system with a Waters 996 ultraviolet (UV-PDA) detector operated at 210 nm and a Waters 717 autoinjector. The sample passed through a Dionex ISA-6 analytical column, 250 X 9 mm and a Hamilton PRP guard column. All injection volumes were 10 µl. The mobile phase was 0.2 mM H_2SO_4, pH 2.2, pumped at 0.8 ml/min isocratically.

Aloin A analysis was performed using a Waters Millennium HPLC system with a Waters 996 ultraviolet (UV-PDA) detector operated at 360 nm and a Waters 717 autoinjector. The sample passed through a Phenomenex 5 µm ODS3 prodigy analytical column, 250 X 4.6 mm and an Upchurch 20 x 2.0 mm guard column filled with Supelco pellicular C18. All injection volumes were 10 µl. The mobile phase was 25% ACN/75% H_2O, 0.01M NaH_2PO_4, pH 4.4, pumped at 1.0 ml/min isocratically.

The average molecular weight of the polysaccharide content of each test article was determined by size exclusion HPLC with Rayleigh light-scattering detection. Samples of the test article (n=4) and Polymer Standards Service GmbH Dextran MW reference materials were solubilized in mobile phase at ~15 mg/ml and filtered through 0.45 µm nylon syringe filters prior to HPLC analysis. Analysis was conducted using a Waters HPLC system consisting of a 717 Autosampler, a 600E Pump and Controller, a 996 Photodiode Array detector (PDA), and 410 Differential Refractometer (RI). A Precision Detectors Inc. (PDI) PD2000/DLS Laser Light Scattering Detection System was installed in the Waters 410 unit. The autosampler, pump, and PDA were controlled using Waters Millennium[32] software. The mobile phase and diluent for the test article samples was 50 mM NaH_2PO_4, 0.05% NaN_3. A YMC 300A Silica Diol (8 x 300 mm) analytical column was utilized with a flow rate of 0.9 ml/min. The data was attained from RI and laser light-scattering 90° detectors using PDI PrecisionAcquire[32] Acquisition Program and data analysis was performed using PDI Discovery[32] Light Scattering Analysis Software.

Homogeneity of a 3% solution of WLN in water was determined based on aloin A content as the 0 h time point of a stability study. Stability of aloin A was assessed for up to 96 h storage at room temperature and 2-8°C for *Aloe barbadensis* WLN test article mixed in water at 0.5, 1.0, 1.5, 2.0 and 3.0% concentrations. WLN Lot 05001A was mixed in water by Diet Preparation and delivered to Chemistry on 3/23/05 as SCR# 2142 99 00649. Aloin A contents were determined per Chemistry SOP No. 532. Results of the homogeneity evaluation were 170 ± 2 µg/ml, %CV=1.4 for aloin A (98.6% of Target) and 5860 ± 80 µg/ml, CV=1.4 for malic acid (98.7% of Target).

Glycosyl linkage analysis was performed on each test article by the Complex Carbohydrate Research Center of the University of Georgia in October of 2007. The samples were all fairly similar in that 4 mannopyranose and terminal glucose were the most prominent linkages present. Samples were permethylated, depolymerized, reduced, and acetylated; and the resultant partially methylated alditol acetates were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard 5890 GC interfaced to a 5970 MSD (mass selective detector, electron impact ionization mode). Separation was performed on a 30 m Supelco 2330 bonded phase fused silica capillary column.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The Bionetics, Inc. Diet Preparation support group prepared the dosed water formulations. For the 14-day studies, aqueous 3% (wt/wt) master batch formulations of the lyophilized Aloe vera extracts were prepared on a daily basis (Monday - Sunday). Due to the water-binding capacity of the Aloe vera gel, dissolution of the test articles in water was performed by gentle mixing with a planetary mixer (Hobart, model KSM90) overnight in a walk-in cooler that was maintained at 4° C. For the 13-week and 2-year studies, aqueous 3% (wt/wt) master batch formulations of the lyophilized Aloe vera whole leaf extract were prepared three times weekly (Monday, Wednesday, and Friday) and twice weekly (Monday and Thursday), respectively. The dissolution of the test article in water was achieved by stirring for 2 h (Lightnin mixer, model EV1P25, Baldor Electric Co., Fort Smith, AR) in a walk-in cooler that was maintained at 4° C. Millipore 0.2 µm-filtered tap water served as the diluent for the dosed water formulations and as

the control group treatment. Formulations were stored at 4° C to ensure the quality and stability of Aloe vera extracts that were administered to animals.

Samples of the control and each level of dosed water for each extract were collected from each mix and submitted to the Chemistry Support Group in the Division of Biochemical Toxicology at NCTR. Dose certifications were conducted twice weekly for the 14-day studies and weekly for the 13-week and 2-year studies by HPLC analysis in a random order for each dose level. The detection and quantification of malic acid and aloin A in the dosed water samples were compared to targeted concentrations of malic acid and aloin A obtained from the homogeneity test results on the different lots of the irradiated Aloe vera extracts. HPLC analyses were unable to detect malic acid or aloin A in control water samples.

Dose certification analyses were performed for solutions of WLN Test Article prepared and submitted for analysis by Diet Preparation. All analyses were performed using the Division of Chemistry SOP No. 532. For each date sampled for dose certification, SCR Numbers were assigned in ascending order for 0, 0.5, 1.0, 1.5, 2.0, and 3.0% (by weight) concentrations of specified Lots of WLN Test Article in solution, which resulted in the listed Target values for malic acid and aloin A.

TABLE H1
Preparation and Storage of Dose Formulations in the Drinking Water Studies of Aloe vera Extracts

14-Day Studies	13-Week Studies	2-Year Studies
Preparation		
Aqueous 3% (wt/wt) master batch formulations of the lyophilized Aloe vera extracts were prepared on a daily basis. Dissolution of the test articles in water was performed by gentle mixing with a planetary mixer overnight at 4° C.	Aqueous 3% (wt/wt) master batch formulations of the lyophilized Aloe vera whole leaf extract were prepared three times weekly. The dissolution of the test article in water was achieved by stirring for 2 h at 4° C.	Aqueous 3% (wt/wt) master batch formulations of the lyophilized Aloe vera whole leaf extract were prepared twice weekly. The dissolution of the test article in water was achieved by stirring for 2 h at 4° C.
Chemical Lot Numbers		
Aloe vera gel extract: 020318AG, 060308AG, 020810AG, and 022308AG Aloe vera whole leaf extract: 020228ND, 060308ND, and 020928ND Aloe vera decolorized whole leaf extract: 020223AC, 060308AC, and 020916AC	Aloe vera whole leaf extract: 042803ND, 032606ND, 081303ND, 082203ND, 090803ND, 093003ND, and 100203ND	Aloe vera whole leaf extract: 041214ND, 040930ND, 041007ND, 041119ND, and 041210ND
Maximum Storage Time		
	72 h storage at room temperature or 96 hours storage at 2-8°C	72 h storage at room temperature or 96 hours storage at 2-8°C
Storage Conditions		
Stored in 250 ml high density polyethylene water bottles with capped sipper tubes, packaged into stainless steel bottle racks, and wrapped in cellophane at 4°C.	Stored in 250 ml high density polyethylene water bottles with capped sipper tubes, packaged into stainless steel bottle racks, and wrapped in cellophane at 4°C.	Stored in 250 ml high density polyethylene water bottles with capped sipper tubes, packaged into stainless steel bottle racks, and wrapped in cellophane at 4°C.
Study Laboratory		
National Center for Toxicological Research (Jefferson, Arkansas)	National Center for Toxicological Research (Jefferson, Arkansas)	National Center for Toxicological Research (Jefferson, Arkansas)

TABLE H2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Day Studies of Aloe vera Extracts

Date Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
Gel Extract						
September 2, 2002	0	<LOQ[a]		0	<LOQ[a]	
	1060	790 ± 10	74.6	5.55	4.8 ±0.2	87.0
	2120	1730 ± 20	81.6	11.1	10.4 ± 0.2	93.3
	3180	2640 ± 10	83.0	16.7	15.9 ± 0.3	95.4
	4240	3680 ± 70	86.8	22.2	23.1 ± 0.6	104
	6360	6050 ± 180	95.1	33.3	38.0 ± 0.9	114
September 4, 2002	0	<LOQ		0	<LOQ	
	1060	790 ± 30	74.2	5.55	5.7 ± 0.2	102
	2120	1770 ± 80	83.3	11.1	11.5 ± 1.3	104
	3180	2550 ± 30	80.1	16.7	17.5 ± 0	105
	4240	3600 ± 90	85.0	22.2	23.7 ± 0.1	107
	6360	6180 ± 170	97.2	33.3	38.2 ± 0.3	115
September 9, 2002	0	<LOQ		0	<LOQ	
	1060	890 ± 0	84.1	5.55	5.5 ± 0	99.6
	2120	1610 ± 40	76.0	11.1	11.8 ± 0.2	107
	3180	2610 ± 160	81.9	16.7	18.2 ± 0.4	109
	4240	3540 ± 70	83.4	22.2	25.6 ± 1.0	115
	6360	5500 ± 40	86.5	33.3	35.7 ± 0.3	107
September 11, 2002	2120	1610 ± 10	76.2	11.1	11.2 ± 0.1	101
	3180	2710 ± 20	85.3	16.7	16.8 ± 0.1	101
	4240	3790 ± 0	89.5	22.2	23.4 ± 0.1	105
	6360	6250 ± 160	98.3	33.3	38.8 ± 0.5	117
September 16, 2002	0	<LOQ		0	<LOQ	
	1060	720 ± 10	68.3	5.55	4.8 ± 0.2	87.1
	2120	1640 ± 40	77.5	11.1	10.5 ± 0.1	94.5
	3180	2250 ± 0	70.8	16.7	16.1 ± 0.4	96.9
	4240	3400 ± 180	80.2	22.2	22.2 ± 0	99.9
	6360	5900 ± 30	92.7	33.3	36.1 ± 0.4	106
September 18, 2002	1060	800 ± 40	75.7	5.55	5.1 ± 0	91.9
	2120	1700 ± 60	80.0	11.1	10.4 ± 0.2	94.0
	3180	2380 ± 100	75.0	16.7	15.6 ± 0.7	93.6
	4240	3630 ± 300	85.6	22.2	23.2 ± 0.1	105
	6360	5920 ± 200	93.1	33.3	40.4 ± 0.8	121
September 23, 2002	0	<LOQ		0	<LOQ	
	1060	830 ± 60	78.7	5.55	4.6 ± 0	83.5
	2120	1740 ± 50	82.0	11.1	10.6 ± 0.2	95.7
	3180	2830 ± 290	89.0	16.7	16.3 ± 0.7	97.6
	4240	4010 ± 10	94.6	22.2	25.5 ± 0.4	115
October 31, 2002	0	<LOQ		0	<LOQ	
	870	650 ± 30	75.2	6.05	4.9 ± 0.2	81.1
	1740	1500 ± 40	86.1	12.1	11.6 ± 0.2	95.9
	2610	2220 ± 80	85.2	18.2	17.3 ± 0.1	95.3
	3480	2900 ± 110	83.3	24.4	22.8 ± 0.8	94.1
	5220	5080 ± 110	97.3	36.3	37.0 ± 1.3	102
November 4, 2002	0	<LOQ		0	<LOQ	
	870	800 ± 10	91.8	6.05	5.2 ± 0.3	86.3
	1740	1730 ± 70	99.4	12.1	11.0 ± 0.1	90.7
	2610	2580 ± 60	99.0	18.2	16.7 ± 0.3	92.2
	3480	3790 ± 200	109	24.4	26.5 ± 0.3	110
	5220	5970 ± 140	114	36.3	42.0 ± 0.2	116

TABLE H2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Day Studies of Aloe vera Extracts (continued)

Date Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
Gel Extract (continued)						
November 6, 2002	0	<LOQ		0	<LOQ	
	870	800 ± 10	91.7	6.05	4.9 ± 0.0	81.4
	1740	1670 ± 0	96.2	12.1	10.8 ± 0.3	89.1
	2610	2630 ± 40	101	18.2	18.4 ± 0.1	101
	3480	3590 ± 10	103	24.4	25.1 ± 0.3	104
	5220	6130 ± 280	118	36.3	41.5 ± 1.3	114
November 13, 2002	0	<LOQ		0	<LOQ	
	870	760 ± 40	86.9	6.05	4.9 ± 0.0	81.3
	1740	1590 ± 30	91.6	12.1	10.3 ± 0.1	84.8
	2610	2470 ± 50	94.7	18.2	16.3 ± 0.1	89.8
	3480	3450 ± 10	99.3	24.4	24.8 ± 0.8	103
	5220	5930 ± 70	114	36.3	39.7 ± 0.7	109
December 9, 2002	0	<LOQ		0	<LOQ	
	605	390 ± 40	64.7	6.9	6.9 ± 2	101
	1210	870 ± 30	71.7	13.7	13.8 ± 0.1	101
	1815	1340 ± 20	73.7	20.6	20.4 ± 0.3	99.1
	2420	1790 ± 30	73.8	27.4	28.8 ± 1.1	105
	3630	2820 ± 10	77.6	41.1	43.3 ± 0.6	105
December 11, 2002	0	<LOQ		0	<LOQ	
	605	460 ± 10	76.0	6.9	6.7 ± 0.1	98.1
	1210	960 ± 10	79.0	13.7	14.3 ± 0.2	104
	1815	1360 ± 30	74.9	20.6	21.5 ± 0.1	105
	2420	1920 ± 40	79.2	27.4	29.3 ± 0.0	107
	3630	2990 ± 40	82.4	41.1	46.0 ± 0.9	112
December 16, 2002	0	<LOQ		0	<LOQ	
	605	460 ± 10	75.9	6.9	6.7 ± 0.2	98.4
	1210	940 ± 20	77.8	13.7	14.1 ± 0.3	103
	1815	1470 ± 70	81.1	20.6	21.1 ± 0.5	103
	2420	1940 ± 40	80.0	27.4	29.6 ± 0.2	108
	3630	3040 ± 40	83.9	41.1	44.5 ± 0.5	108
December 18, 2002	0	<LOQ		0	<LOQ	
	605	430 ± 20	71.8	6.9	6.3 ± 0.1	92.3
	1210	880 ± 20	72.4	13.7	14.1 ± 0.3	103
	1815	1360 ± 50	75.2	20.6	20.6 ± 0.0	100
	2420	1950 ± 90	80.5	27.4	28.9 ± 0.4	106
	3630	2900 ± 0	79.9	41.1	44.7 ± 0.8	109
June 26, 2003	0	<LOQ		0	<LOQ	
	605	340 ± 10	56.5	6.85	5.9 ± 0.2	86.5
	1210	610 ± 20	50.1	13.7	12.5 ± 0.2	91.5
	1815	1090 ± 60	60.0	20.6	20.3 ± 0.6	98.7
	2420	1420 ± 110	58.7	27.4	26.2 ± 1.5	95.7
	3630	2280 ± 110	62.9	41.1	38.5 ± 2.4	93.7
July 1, 2003	0	<LOQ		0	<LOQ	
	605	410 ± 50	68.1	6.85	7.0 ± 0.6	102
	1210	800 ± 40	65.7	13.7	14.3 ± 0.3	105
	1815	1260 ± 60	69.5	20.6	20.3 ± 0.7	98.8
	2420	1700 ± 80	70.3	27.4	27.3 ± 1.6	99.6
	3630	2540 ± 160	70.0	41.1	43.3 ± 2.7	105
July 3, 2003	0	<LOQ		0	<LOQ	
	605	390 ± 0	64.7	6.85	6.9 ± 0.1	101
	1210	830 ± 20	68.7	13.7	14.8 ± 0.2	108
	1815	1320 ± 10	72.7	20.6	20.8 ± 0	102
	2420	1880 ± 30	77.7	27.4	29.2 ± 0.2	107
	3630	3010 ± 100	82.9	41.1	48.4 ± 2.1	118

TABLE H2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Day Studies of Aloe vera Extracts (continued)

Date Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
Gel Extract (continued)						
July 8, 2003	0	<LOQ		0	<LOQ	
	605	380 ± 0	62.8	6.85	6.9 ± 0.3	101
	1210	780 ± 50	64.1	13.7	13.9 ± 0.5	102
	1815	1200 ± 40	65.9	20.6	20.3 ± 0.4	98.8
	2420	1590 ± 130	65.7	27.4	27.1 ± 1.2	99.1
	3630	2430 ± 100	66.9	41.1	39.6 ± 2.2	96.4
Whole Leaf Decolorized						
September 2, 2002	1215	1090 ± 10	89.6	0.75	0.7 ± 0	92.5
	2430	2140 ± 10	87.9	1.49	1.3 ± 0	85.7
	3645	3220 ± 40	88.5	2.24	1.9 ± 0	84.4
	4860	4350 ± 10	89.5	2.98	2.5 ± 0	83.3
	7290	6640 ± 80	91.1	4.47	3.7 ± 0	81.8
September 4, 2002	1215	1030 ± 50	84.8	0.75	0.7 ± 0	91.3
	2430	2100 ± 20	86.4	1.49	1.4 ± 0	95.9
	3645	3070 ± 80	84.2	2.24	2.1 ± 0	92.7
	4860	4300 ± 130	88.5	2.98	2.7 ± 0	92.0
	7290	6490 ± 250	89.0	4.47	4.1 ± 0.2	90.7
September 9, 2002	1215	1080 ± 30	89.1	0.75	0.8 ± 0	107
	2430	2290 ± 20	94.1	1.49	1.6 ± 0	107
	3645	3170 ± 60	86.9	2.24	2.2 ± 0	99.9
	4860	4620 ± 170	95.0	2.98	3.0 ± 0	101
	7290	6980 ± 190	95.7	4.47	4.5 ± 0	99.7
September 11, 2002	1215	1030 ± 50	85.2	0.75	0.8 ± 0	102
	2430	2120 ± 30	87.4	1.49	1.5 ± 0.1	97.9
	3645	3280 ± 100	90.1	2.24	2.0 ± 0	89.6
	4860	4510 ± 160	92.7	2.98	2.7 ± 0.1	92.2
	7290	6710 ± 100	92.1	4.47	4.3 ± 0	95.5
September 16, 2002	1215	970 ± 30	80.1	0.75	0.7 ± 0	91.6
	2430	2100 ± 80	86.5	1.49	1.4 ± 0	91.6
	3645	3050 ± 20	83.7	2.24	1.9 ± 0	85.8
	4860	4260 ± 180	87.7	2.98	2.5 ± 0	83.1
	7290	6590 ± 240	90.4	4.47	3.8 ± 0.1	84.9
September 18, 2002	0	<LOQ		0	<LOQ	
	1215	950 ± 10	77.9	0.75	0.7 ± 0	93.7
	2430	2050 ± 30	84.5	1.49	1.3 ± 0.1	88.5
	3645	3220 ± 180	88.3	2.24	2.0 ± 0	91.0
	4860	4200 ± 10	86.4	2.98	2.7 ± 0.1	91.9
	7290	6460 ± 100	88.6	4.47	4.2 ± 0	92.9
September 23, 2002	1215	920 ± 0	75.9	0.75	0.5 ± 0	73.3
	2430	1890 ± 20	77.9	1.49	1.2 ± 0	77.6
	3645	3180 ± 100	87.3	2.24	1.9 ± 0.1	86.3
	4860	4260 ± 90	87.6	2.98	2.8 ± 0.1	92.5
	7290	6130 ± 130	84.0	4.47	3.9 ± 0	86.3
January 9, 2003	0	<LOQ		0	<LOQ	
	1240	1290 ± 40	104	0.32	0.3 ± 0	99.1
	2480	2600 ± 40	105	0.63	0.6 ± 0	100
	3720	3660 ± 20	98.5	0.95	0.9 ± 0	94.3
	4960	4890 ± 50	98.6	1.26	1.2 ± 0	95.7
	7440	7500 ± 60	101	1.89	1.7 ± 0	90.6

TABLE H2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Day Studies of Aloe vera Extracts (continued)

Date Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
Whole Leaf Decolorized (continued)						
January 13, 2003	0	<LOQ		0	<LOQ	
	1240	1270 ± 10	103	0.32	0.3 ± 0	85.5
	2480	2260 ± 40	91.2	0.63	0.7 ± 0	108
	3720	3490 ± 10	93.9	0.95	0.9 ± 0	95.0
	4960	4740 ± 130	95.5	1.26	1.3 ± 0	99.4
	7440	7420 ± 70	99.7	1.89	2.0 ± 0.1	103
January 15, 2003	0	<LOQ		0	<LOQ	
	1240	1330 ± 10	107	0.32	0.3 ± 0	92.3
	2480	2420 ± 20	97.7	0.63	0.7 ± 0	111
	3720	3590 ± 100	96.5	0.95	0.9 ± 0	100
	4960	4850 ± 0	97.8	1.26	1.3 ± 0	100
	7440	7530 ± 50	101	1.89	2.0 ± 0	108
January 20, 2003	0	<LOQ		0	<LOQ	
	1240	1220 ± 40	98.1	0.32	0.3 ± 0	83.0
	2480	2460 ± 50	99.3	0.63	0.6 ± 0	89.0
	3720	3640 ± 40	98.0	0.95	0.9 ± 0	91.4
	4960	4940 ± 40	99.6	1.26	1.3 ± 0	102
	7440	7570 ± 100	102	1.89	1.9 ± 0.1	101
January 22, 2003	0	<LOQ		0	<LOQ	
	1240	1300 ± 50	105	0.32	0.2 ± 0	69.8
	2480	2400 ± 10	97.0	0.63	0.5 ± 0	82.2
	3720	3610 ± 20	97.0	0.95	0.9 ± 0	93.5
	4960	4900 ± 40	98.8	1.26	1.3 ± 0	105
	7440	7360 ± 80	99.0	1.89	1.7 ± 0.1	91.6
June 17, 2003	0	<LOQ		0	<LOQ	
	1240	1450 ± 90	117	0.32	0.2 ± 0	75.3
	2480	2770 ± 60	112	0.63	0.4 ± 0	69.2
	3720	3800 ± 80	102	0.95	0.7 ± 0	78.3
	4960	4980 ± 0	100	1.3	1.0 ± 0	77.0
	7440	7350 ± 70	98.8	1.9	1.3 ± 0	69.5
June 19, 2003	0	<LOQ		0	<LOQ	
	1240	780 ± 30	62.6	0.32	0.2 ± 0.1	64.6
	2480	2410 ± 40	97.2	0.63	0.4 ± 0	67.1
	3720	3490 ± 40	93.9	0.95	0.7 ± 0.1	75.6
	4960	4740 ± 10	95.6	1.3	1.0 ± 0	78.8
	7440	7210 ± 100	96.9	1.9	1.4 ± 0	75.4
June 24, 2003	0	<LOQ		0	<LOQ	
	1240	1100 ± 50	88.8	0.32	0.4 ± 0	116
	2480	2210 ± 50	89.0	0.63	0.8 ± 0.1	131
	3720	3200 ± 110	86.1	0.95	0.8 ± 0	80.0
	4960	4440 ± 0	89.5	1.3	1.0 ± 0.1	80.3
	7440	6890 ± 70	92.6	1.9	1.9 ± 0	100
June 26, 2003	0	<LOQ		0	<LOQ	
	1240	1210 ± 30	97.9	0.32	0.2 ± 0	76.5
	2480	2280 ± 60	91.8	0.63	0.7 ± 0	115
	3720	3390 ± 60	91.0	0.95	0.8 ± 0	80.4
	4960	4490 ± 20	90.6	1.3	1.1 ± 0	87.7
	7440	6940 ± 110	93.2	1.9	1.7 ± 0.1	90.0

TABLE H2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Day Studies of Aloe vera Extracts (continued)

Date Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
Whole Leaf Non-decolorized						
September 2, 2002	970	840 ± 10	86.4	70.4	57.2 ± 0.5	81.2
	1940	1770 ± 0	91.2	141	120 ± 1	85.1
	2910	2700 ± 30	92.7	212	189 ± 1	89.3
	3880	3380 ± 30	87.1	282	236 ± 2	83.7
	5820	5580 ± 20	95.8	422	388 ± 3	91.8
September 4, 2002	970	880 ± 0	90.3	70.4	65 ± 0.6	92.4
	1940	1800 ± 30	92.8	141	130 ± 1	92.2
	2910	2710 ± 40	93.1	212	197 ± 2	93.0
	3880	3750 ± 50	96.8	282	269 ± 10	95.6
	5820	5830 ± 130	100	422	405 ± 5	95.9
September 9, 2002	970	910 ± 10	93.4	70.4	64.0 ± 1.2	90.9
	1940	1820 ± 40	94.0	141	132 ± 0	93.8
	2910	2760 ± 90	94.7	212	198 ± 1	93.6
	3880	3790 ± 10	97.7	282	273 ± 2	96.9
	5820	5930 ± 0	102	422	409 ± 3	96.9
September 11, 2002	970	950 ± 20	97.9	70.4	67.8 ± 1.7	96.3
	1940	1900 ± 10	98.1	141	137 ± 2	97.4
	2910	2810 ± 0	96.5	212	206 ± 1	97.5
	3880	3820 ± 10	98.4	282	275 ± 4	97.6
	5820	5920 ± 30	102	422	406 ± 6	96.2
September 16, 2002	970	910 ± 10	93.4	70.4	63.3 ± 0.8	89.9
	1940	1790 ± 30	92.1	141	129 ± 2	91.3
	2910	2770 ± 30	95.1	212	198 ± 6	93.5
	3880	3710 ± 10	95.7	282	269 ± 3	95.4
	5820	5690 ± 0	97.7	422	394 ± 2	93.2
September 18, 2002	970	890 ± 30	92.0	70.4	63.6 ± 0.4	90.4
	1940	1750 ± 0	90.3	141	135 ± 0	95.6
	2910	2730 ± 60	93.9	212	210 ± 1	99.1
	3880	3700 ± 40	95.4	282	279 ± 4	99.0
	5820	5650 ± 10	97.2	422	402 ± 7	95.1
September 23, 2002	1940	1640 ± 0	84.6	141	118 ± 1	84.1
	2910	2590 ± 40	89.1	212	187 ± 1	88.2
	3880	3650 ± 50	94.2	282	253 ± 1	89.8
	5820	5420 ± 90	93.1	422	371 ± 0	87.9
December 5, 2002	0	<LOQ		0	<LOQ	
	970	940 ± 40	96.6	78	67.8 ± 1.3	86.9
	1940	1840 ± 70	94.9	156	140 ± 0	91.9
	2910	2720 ± 20	93.4	234	212 ± 2	90.3
	3880	3730 ± 70	96.0	312	287 ± 9	91.9
	5820	5670 ± 60	97.4	468	423 ± 5	90.3
December 9, 2002	0	<LOQ		0	<LOQ	
	970	940 ± 60	96.5	78	66.3 ± 0.1	84.9
	1940	1830 ± 10	94.4	156	142 ± 4	91.2
	2910	2710 ± 10	93.2	234	215 ± 2	91.8
	3880	3700 ± 80	95.3	312	293 ± 1	94.1
	5820	5690 ± 20	97.7	468	435 ± 1	92.9
December 11, 2002	0	<LOQ		0	<LOQ	
	970	910 ± 10	94.2	78	71.0 ± 0.9	91.1
	1940	1890 ± 20	97.2	156	147 ± 1	94.0
	2910	2760 ± 0	94.9	234	225 ± 2	96.3
	3880	3830 ± 0	98.2	312	304 ± 1	97.5
	5820	5850 ± 90	101	468	442 ± 3	94.5

TABLE H2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Day Studies of Aloe vera Extracts (continued)

Date Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
Whole Leaf Non-decolorized (continued)						
December 16, 2002	0	<LOQ		0	<LOQ	
	970	950 ± 60	97.5	78	73.4 ± 1.3	94.1
	1940	1860 ± 20	95.9	156	152 ± 1	97.2
	2910	2850 ± 20	97.9	234	230 ± 2	98.2
	3880	3820 ± 20	98.5	312	306 ± 0	98.0
	5820	5860 ± 10	101	468	452 ± 2	96.5
December 18, 2002	0	<LOQ		0	<LOQ	
	970	880 ± 30	91.2	78	71.9 ± 0.2	92.2
	1940	1840 ± 10	95.0	156	150 ± 1	96.4
	2910	2730 ± 10	94.0	234	218 ± 5	93.0
	3880	3730 ± 20	96.0	312	300 ± 0	96.0
	5820	5700 ± 20	97.9	468	442 ± 3	94.5
June 12, 2003	0	<LOQ		0	<LOQ	
	970	960 ± 10	99.4	78	65.9 ± 1.4	84.5
	1940	1960 ± 20	101	156	130 ± 0	83.6
	2910	2840 ± 10	97.5	234	199[b]	85.1
	3880	3870 ± 50	99.7	312	270 ± 5	86.5
	5820	6030 ± 150	104	468	422 ± 0	90.1
June 17, 2003	0	<LOQ		0	<LOQ	
	970	860 ± 40	88.6	78	62.1 ± 3.7	79.6
	1940	1840 ± 60	94.9	156	132 ± 6	84.3
	2910	2710 ± 60	93.3	234	198[b]	84.4
	3880	3680 ± 90	94.9	312	271 ± 6	86.7
	5820	5650 ± 80	97.1	468	400 ± 6	85.4
June 19, 2003	0	<LOQ		0	<LOQ	
	970	970 ± 10	99.7	78	73.7 ± 0	94.5
	1940	1980 ± 30	102	156	152 ± 1	97.6
	2910	2910 ± 40	100	234	220[b]	94.2
	3880	3830 ± 80	98.7	312	295 ± 15	94.6
	5820	6080 ± 40	105	468	444 ± 12	94.9
June 24, 2003	0	<LOQ		0	<LOQ	
	970	830 ± 70	85.1	78	46.6 ± 2.2	59.7
	1940	1710 ± 10	88.0	156	95.6 ± 2.0	61.0
	2910	2550 ± 30	87.6	234	148 ± 4	63.2
	3880	3510 ± 60	90.6	312	196 ± 6	62.8
	5820	4870 ± 70	83.6	468	292 ± 36	62.4

[a] The limits of quantitation were estimated to be 100 ppm malic acid and 0.1 ppm aloin A.
[b] n=1

TABLE H3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 13-Week Studies of Aloe vera Whole Leaf Extract

	Malic acid			Aloin A		
Date Sampled	**Target (ppm)**	**Determined (ppm)**	**% Target**	**Target (ppm)**	**Determined (ppm)**	**% Target**
November 14, 2003	0	<LOQ[a]		0	<LOQ[a]	
	1830	1700 ± 110	92.9	129	134 ± 7	104
	3660	3660 ± 90	100	258	273 ± 8	106
	5490	5400 ± 30	98.4	387	411 ± 5	106
November 20, 2003	0	<LOQ		0	<LOQ	
	1830	2000 ± 490	110	129	162 ± 40	126
	3660	3290 ± 10	89.9	258	257 ± 8	99.6
	5490	5510 ± 0	100	387	397 ± 10	103
November 26, 2003	0	<LOQ		0	<LOQ	
	1830	1710 ± 20	93.2	129	132 ± 0	102
	3660	3430 ± 130	93.6	258	267 ± 7	103
	5490	5330 ± 40	97.2	387	392 ± 4	101
December 1, 2003	0	<LOQ		0	<LOQ	
	1830	1760 ± 70	95.9	129	139 ± 1	108
	3660	3500 ± 20	95.7	258	277 ± 1	107
	5490	5230 ± 200	95.3	387	399 ± 8	103
December 8, 2003	0	<LOQ		0	<LOQ	
	1830	1730 ± 50	94.7	129	133 ± 2	103
	3660	3100 ± 20	84.8	258	226 ± 1	87.6
	5490	4640 ± 210	84.6	387	351 ± 14	90.7
December 19, 2003	0	<LOQ		0	<LOQ	
	1830	1740 ± 20	95.0	129	130 ± 0	101
	3660	3460 ± 90	94.5	258	259 ± 3	100
	5490	4380 ± 210	98.0	387	401 ± 11	104
December 22, 2003	0	<LOQ		0	<LOQ	
	1830	1800 ± 20	98.2	129	133 ± 3	103
	3660	3480 ± 20	95.1	258	263 ± 1	102
	5490	5400 ± 150	98.3	387	398 ± 6	103
January 2, 2004	0	<LOQ		0	<LOQ	
	1830	1760 ± 0	96.3	129	146 ± 3	113
	3660	3550 ± 70	97.0	258	288 ± 6	111
	5490	5470 ± 70	99.7	387	430 ± 4	111
January 7, 2004	0	<LOQ		0	<LOQ	
	1830	1760 ± 0	95.5	129	145 ± 0	112
	3660	3550 ± 70	96.3	258	281 ± 10	109
	5490	5410 ± 250	98.6	387	443 ± 4	114
January 14, 2004	0	<LOQ		0	<LOQ	
	1830	1760 ± 40	96.4	129	138 ± 1	107
	3660	3610 ± 0	98.7	258	276 ± 3	107
	5490	5270 ± 70	96.0	387	406 ± 6	105
January 19, 2004	0	<LOQ		0	<LOQ	
	1830	1730 ± 10	94.4	129	136 ± 3	105
	3660	3440 ± 60	93.9	258	264 ± 1	102
	5490	5210 ± 10	94.8	387	388 ± 2	100
January 28, 2004	0	<LOQ		0	<LOQ	
	1830	1780 ± 20	97.3	129	136 ± 2	105
	3660	3430 ± 70	93.7	258	280 ± 9	109
	5490	5230 ± 100	95.3	387	414 ± 12	107
February 2, 2004	0	<LOQ		0	<LOQ	
	1830	1710 ± 10	93.6	129	138 ± 1	107
	3660	3210 ± 80	87.6	258	248 ± 9	96.1
	5490	5140 ± 140	93.7	387	415 ± 3	107
February 13, 2004	0	<LOQ		0	<LOQ	
	1830	1710 ± 0	93.7	129	150 ± 2	116
	3660	3470 ± 10	87.6	258	284 ± 3	110
	5490	5350 ± 60	97.5	387	430 ± 3	111

TABLE H3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 13-Week Studies of Aloe vera Whole Leaf Extract

	Malic acid			Aloin A		
Date Sampled	**Target (ppm)**	**Determined (ppm)**	**% Target**	**Target (ppm)**	**Determined (ppm)**	**% Target**
February 17, 2004	0	<LOQ		0	<LOQ	
	1830	1720 ± 0	93.8	129	129 ± 3	100
	3660	3390 ± 130	92.7	258	255 ± 3	98.8
	5490	5190 ± 10	94.5	387	377 ± 2	97.4
February 25, 2004	0	<LOQ		0	<LOQ	
	3660	3470 ± 0	94.9	258	255 ± 0	98.8
March 3, 2004	0	<LOQ		0	<LOQ	
	3660	3480 ± 30	95.2	258	253 ± 2	98.1
March 12, 2004	0	<LOQ		0	<LOQ	
	3660	3660 ± 50	99.9	258	265 ± 1	103
March 17, 2004	0	<LOQ		0	<LOQ	
	3660	3370 ± 120	92.1	258	267 ± 11	103
March 22, 2004	0	<LOQ		0	<LOQ	
	3660	3460 ± 10	94.5	258	273 ± 1	106
March 31, 2004	0	<LOQ		0	<LOQ	
	3660	3610 ± 80	98.6	258	303 ± 0	117
April 5, 2004	0	<LOQ		0	<LOQ	
	3660	3380 ± 40	92.5	258	268 ± 3	104

[a] The limits of quantitation were estimated to be 100 ppm malic acid and 0.1 ppm aloin A.

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract[a]

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
April 25, 2005	0	<LOQ[b]		0	<LOQ	
	990	880 ± 80	89.7	28.7	28.8 ± 0.3	101
	1980	2070 ± 120	104	57.3	60.2 ± 0.4	105
	2970	3040 ± 140	102	86.0	92.7 ± 1.4	108
	3960	3800 ± 60	95.8	115	124 ± 1	109
	5940	5710 ± 170	96.1	172	177 ± 4	103
April 28, 2005	0	<LOQ		0	<LOQ	
	990	880 ± 35	88.9	28.7	30.0 ± 0.1	105
	1980	1920 ± 50	97.0	57.3	59.7 ± 0.4	104
	2970	2850 ± 60	95.8	86.0	91.4 ± 0.4	106
	3960	3910 ± 30	98.7	115	123 ± 0	107
	5940	5880 ± 240	98.9	172	180 ± 1	105
May 5, 2005	0	<LOQ		0	<LOQ	
	990	892 ± 7	90.1	28.7	28.6 ± 0.2	99.8
	1980	2020 ± 30	102	57.3	59.2 ± 0.5	103
	2970	2850 ± 40	95.9	86.0	90.3 ± 1.0	105
	3960	3930 ± 40	99.2	115	124 ± 0	108
	5940	5740 ± 180	96.6	172	180 ± 3	105
May 12, 2005	0	<LOQ		0	<LOQ	
	990	907 ± 26	91.6	28.7	30.4 ± 1.7	106
	1980	1990 ± 40	101	57.3	60.3 ± 0.1	105
	2970	2780 ± 50	93.6	86.0	93.2 ± 1.6	109
	3960	3770 ± 60	95.3	115	124 ± 3	108
	5940	5520 ± 210	92.9	172	183 ± 2	106
May 16, 2005	0	<LOQ		0	<LOQ	
	990	914 ± 31	92.3	28.7	30.7 ± 0.4	107
	1980	1930 ± 60	97.6	57.3	61.5 ± 0.4	107
	2970	2770 ± 30	93.3	86.0	92.6 ± 0.7	108
	3960	3780 ± 70	95.6	115	125 ± 1	109
	5940	5580 ± 10	94.0	172	185 ± 1	108
May 23, 2005	0	<LOQ		0	<LOQ	
	990	989 ± 22	99.9	28.7	32.2 ± 0.4	113
	1980	2000 ± 60	101	57.3	64.7 ± 1.0	113
	2970	2940 ± 40	98.8	86.0	101 ± 1	118
	3960	3990 ± 20	101	115	136 ± 1	118
	5940	5650 ± 100	95.2	172	201 ± 3	117
June 2, 2005	0	<LOQ		0	<LOQ	
	990	989 ± 22	90.2	28.7	32.2 ± 0.4	118
	1980	2000 ± 60	96.2	57.3	64.7 ± 1.0	120
	2970	2780 ± 40	93.5	86.0	97.8 ± 1.7	114
	3960	3780 ± 10	95.3	115	132 ± 1	115
	5940	5640 ± 130	95.0	172	203 ± 2	118
June 6, 2005	0	<LOQ		0	<LOQ	
	990	932 ± 14	94.2	28.7	31.9 ± 0.2	111
	1980	1960 ± 40	99.1	57.3	62.8 ± 0.6	110
	2970	2710 ± 90	91.3	86.0	91.3 ± 0.9	106
	3960	3770 ± 50	95.1	115	121 ± 0	106
	5940	5620 ± 40	94.5	172	188 ± 0	110
June 13, 2005	0	<LOQ		0	<LOQ	
	990	941 ± 36	95.1	28.7	34.2 ± 0.1	119
	1980	1840 ± 10	93.1	57.3	68.4 ± 0.2	119
	2970	2770 ± 30	93.3	86.0	102 ± 1	119
	3960	3710 ± 20	93.6	115	133 ± 1	116
	5940	5580 ± 60	94.0	172	203 ± 1	118

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
June 20, 2005	0	<LOQ		0	<LOQ	
	990	922 ± 16	93.1	28.7	33.2 ± 0.2	116
	1980	1860 ± 20	94.1	57.3	66.6 ± 0.3	116
	2970	2790 ± 30	94.0	86.0	99.8 ± 1.2	116
	3960	3710 ± 30	93.8	115	133 ± 2	116
	5940	5670 ± 110	95.5	172	202 ± 4	118
June 30, 2005	0	<LOQ		0	<LOQ	
	990	963 ± 57	97.3	28.7	32.1 ± 0.3	112
	1980	1920 ± 50	96.9	57.3	64.2 ± 0.3	112
	2970	2850 ± 120	96.1	86.0	92.4 ± 0.0	108
	3960	3750 ± 0	94.7	115	126 ± 0	110
	5940	5640 ± 280	95.0	172	189 ± 2	110
July 8, 2005[c]	0	<LOQ		0	<LOQ	
	990	940 ± 5	94.9	28.7	32.6 ± 0.0	114
	1980	1830 ± 90	92.6	57.3	65.2 ± 0.6	114
	2970	2700 ± 120	90.9	86.0	95.9 ± 0.2	112
	3960	3830 ± 50	96.7	115	127 ± 2	111
	5940	5870 ± 180	98.7	172	196 ± 1	114
July 14, 2005	0	<LOQ		0	<LOQ	
	990	1020 ± 20	104	28.7	33.4 ± 0.4	117
	1980	1830 ± 40	92.2	57.3	64.7 ± 0.0	113
	2970	2800 ± 80	94.2	86.0	93.6 ± 1.3	109
	3960	3680 ± 40	92.8	115	126 ± 2	110
	5940	5470 ± 60	92.1	172	189 ± 1	110
July 18, 2005	0	<LOQ		0	<LOQ	
	990	1000 ± 40	101	28.7	30.4 ± 0.5	106
	1980	1830 ± 50	92.7	57.3	61.2 ± 0.2	107
	2970	2720 ± 60	91.7	86.0	91.6 ± 0.3	107
	3960	3740 ± 110	94.5	115	118 ± 1	103
	5940	5680 ± 120	95.5	172	196 ± 2	114
July 25, 2005	0	<LOQ		0	<LOQ	
	990	880 ± 0	89.3	28.7	30.5 ± 0.5	107
	1980	1910 ± 10	96.3	57.3	60.2 ± 0.3	105
	2970	2610 ± 20	87.9	86.0	90.1 ± 0.9	105
	3960	3720 ± 30	94.0	115	124 ± 1	108
	5940	5590 ± 120	94.1	172	197 ± 3	115
August 4, 2005	0	<LOQ		0	<LOQ	
	990	919 ± 2	92.9	28.7	31.5 ± 0.2	110
	1980	1810 ± 20	91.3	57.3	64.3 ± 0.4	112
	2970	2690 ± 60	90.7	86.0	92.1 ± 1.2	107
	3960	3630 ± 120	91.6	115	122 ± 7	107
	5940	5570 ± 140	93.8	172	190 ± 3	110
August 8, 2005	0	<LOQ		0	<LOQ	
	990	1030 ± 0	104	28.7	27.6 ± 0.3	96.4
	1980	1930 ± 10	97.3	57.3	55.9 ± 0.4	97.6
	2970	2740 ± 140	92.2	86.0	78.7 ± 0.4	91.5
	3960	3660 ± 100	92.5	115	105 ± 2	91.6
	5940	5660 ± 50	95.2	172	167 ± 2	96.9

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
August 15, 2005	0	<LOQ		0	<LOQ	
	990	996 ± 3	101	28.7	29.4 ± 0.2	103
	1980	2070 ± 40	105	57.3	58.3 ± 0.7	102
	2970	2720 ± 20	91.6	86.0	84.2 ± 0.4	98.0
	3960	3780 ± 20	95.6	115	113 ± 0	98.6
	5940	5480 ± 110	92.3	172	175 ± 1	102
August 22, 2005	0	<LOQ		0	<LOQ	
	990	929 ± 24	93.9	28.7	32.4 ± 0.0	113
	1980	1950 ± 50	98.2	57.3	64.2 ± 0.2	112
	2970	2830 ± 70	95.3	86.0	97.0 ± 1.3	113
	3960	3910 ± 30	98.7	115	130 ± 6	113
	5940	5670 ± 40	95.5	172	194 ± 1	113
September 1, 2005	0	<LOQ		0	<LOQ	
	990	903 ± 13	91.2	28.7	31.5 ± 0.2	110
	1980	1800 ± 30	90.7	57.3	63.4 ± 0.2	111
	2970	2740 ± 100	92.4	86.0	98.3 ± 0.3	114
	3960	2960 ± 10	74.8	115	102 ± 1	88.6
	5940	5820 ± 20	97.9	172	193 ± 4	112
September 8, 2005	0	<LOQ		0	<LOQ	
	990	954 ± 29	96.4	28.7	32.9 ± 0.0	115
	1980	1800 ± 20	90.8	57.3	66.3 ± 0.3	116
	2970	2800 ± 40	94.2	86.0	102 ± 3	118
	3960	3830 ± 70	96.7	115	135 ± 0	117
	5940	5860 ± 80	98.7	172	196 ± 1	114
September 15, 2005	0	<LOQ		0	<LOQ	
	990	900 ± 11	90.9	28.7	29.8 ± 0.3	104
	1980	1780 ± 20	90.0	57.3	62.7 ± 0.1	109
	2970	2920 ± 20	98.3	86.0	94.8 ± 1.4	110
	3960	4100 ± 60	103	115	128 ± 2	112
	5940	5960 ± 110	100	172	195 ± 2	113
September 22, 2005	0	<LOQ		0	<LOQ	
	990	842 ± 18	85.1	28.7	31.5 ± 0.1	110
	1980	1880 ± 10	94.8	57.3	67.0 ± 1.0	117
	2970	2800 ± 0	94.4	86.0	102 ± 1	119
	3960	3980 ± 30	101	115	134 ± 1	117
	5940	5940 ± 20	100	172	209 ± 2	122
September 26, 2005	0	<LOQ		0	<LOQ	
	990	898 ± 0	90.7	28.7	31.5 ± 0.3	109
	1980	1840 ± 0	93.1	57.3	63.3 ± 0.3	111
	2970	2850 ± 50	96.1	86.0	98.5 ± 0.7	115
	3960	4030 ± 100	102	115	132 ± 0	115
	5940	5680 ± 20	95.6	172	202 ± 3	117
October 3, 2005	0	<LOQ		0	<LOQ	
	990	938 ± 7	94.7	28.7	30.5 ± 0.6	106
	1980	1730 ± 10	87.3	57.3	53.5 ± 0.3	93.4
	2970	2770 ± 30	93.3	86.0	87.0 ± 1.5	101
	3960	3880 ± 40	97.9	115	123 ± 1	107
	5940	5800 ± 140	97.6	172	188 ± 2	109

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
October 10, 2005	0	<LOQ		0	<LOQ	
	990	891 ± 13	94.7	28.7	30.1 ± 0.0	105
	1980	1830 ± 50	87.3	57.3	61.4 ± 0.4	107
	2970	2760 ± 80	93.3	86.0	97.0 ± 0.4	113
	3960	3930 ± 60	97.9	115	128 ± 1	112
	5940	5660 ± 30	97.6	172	191 ± 2	111
October 20, 2005	0	<LOQ		0	<LOQ	
	955	845 ± 33	88.5	32.0	30.0 ± 0.0	93.8
	1910	1730 ± 0	90.7	64.0	61.0 ± 0.4	95.2
	2870	2650 ± 80	92.5	96.0	93.0 ± 0.4	96.9
	3820	4040 ± 110	106	128	126 ± 0	98.4
	5730	5790 ± 140	101	192	193 ± 2	101
October 27, 2005	0	<LOQ		0	<LOQ	
	955	885 ± 11	92.6	32.0	29.0 ± 0.4	90.6
	1910	1830 ± 70	96.0	64.0	59.4 ± 0.4	92.8
	2870	2810 ± 70	98.1	96.0	91.3 ± 1.6	95.1
	3820	3810 ± 80	99.8	128	123 ± 0	95.9
	5730	5640 ± 210	98.5	192	188 ± 2	98.0
November 3, 2005	0	<LOQ		0	<LOQ	
	955	896 ± 23	93.8	32.0	30.7 ± 0.3	95.9
	1910	1810 ± 20	95.0	64.0	63.0 ± 0.3	98.5
	2870	2860 ± 120	99.7	96.0	95.5 ± 0.8	99.4
	3820	3860 ± 20	101	128	128 ± 2	100
	5730	5700 ± 80	99.5	192	196 ± 3	102
November 7, 2005	0	<LOQ		0	<LOQ	
	955	921 ± 26	96.4	32.0	32.7 ± 0.2	102
	1910	1840 ± 50	96.3	64.0	64.0 ± 1.4	100
	2870	2960 ± 170	103	96.0	93.6 ± 0.4	97.5
	3820	3830 ± 100	100	128	126 ± 2	98.6
	5730	5700 ± 60	99.5	192	194 ± 0	101
November 14, 2005	0	<LOQ		0	<LOQ	
	955	884 ± 5	92.5	32.0	32.5 ± 0.4	102
	1910	1850 ± 20	96.7	64.0	65.0 ± 0.0	102
	2870	2760 ± 60	96.2	96.0	99.2 ± 0.9	103
	3820	3900 ± 50	102	128	130 ± 2	101
	5730	5830 ± 30	102	192	204 ± 1	106
November 21, 2005	0	<LOQ		0	<LOQ	
	955	883 ± 16	92.4	32.0	29.6 ± 2.2	92.4
	1910	1790 ± 10	93.9	64.0	63.6 ± 0.9	99.3
	2870	2790 ± 150	97.3	96.0	98.3 ± 1.8	102
	3820	4050 ± 50	106	128	133 ± 0	104
	5730	5750 ± 40	100	192	202 ± 1	105
November 28, 2005	0	<LOQ		0	<LOQ	
	955	873 ± 4	91.4	32.0	29.4 ± 0.4	92.0
	1910	1740 ± 0	91.1	64.0	59.8 ± 0.6	93.5
	2870	2720 ± 80	94.8	96.0	95.2 ± 0.0	99.1
	3820	3930 ± 20	103	128	127 ± 1	99.2
	5730	5800 ± 100	101	192	193 ± 0	100

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
December 8, 2005	0	<LOQ		0	<LOQ	
	955	831 ± 15	87.0	32.0	32.9 ± 0.2	103
	1910	1740 ± 20	91.3	64.0	69.3 ± 1.2	108
	2870	2800 ± 50	97.7	96.0	105 ± 0	109
	3820	3740 ± 70	97.8	128	139 ± 2	109
	5730	5710 ± 120	99.6	192	212 ± 3	110
December 15, 2005	0	<LOQ		0	<LOQ	
	955	877 ± 31	91.8	32.0	31.8 ± 1.8	99.4
	1910	1860 ± 10	97.4	64.0	66.3 ± 0.7	104
	2870	3080 ± 140	107	96.0	102 ± 1	106
	3820	4150 ± 60	109	128	138 ± 0	108
	5730	5740 ± 170	100	192	207 ± 1	108
December 19, 2005	0	<LOQ		0	<LOQ	
	955	888 ± 18	92.9	32.0	28.9 ± 0.4	92.0
	1910	1750 ± 10	91.5	64.0	57.5 ± 0.8	89.8
	2870	2880 ± 10	100	96.0	88.5 ± 0.0	92.1
	3820	4280 ± 50	112	128	121 ± 0	94.3
	5730	5700 ± 10	99.5	192	173 ± 0	90.0
December 26, 2005	0	<LOQ		0	<LOQ	
	955	892 ± 0	93.4	32.0	30.9 ± 0.5	96.6
	1910	1780 ± 30	93.0	64.0	60.3 ± 2.5	94.2
	2870	3060 ± 30	107	96.0	93.4 ± 0.5	97.3
	3820	3250 ± 20	85.2	128	93.5 ± 1.3	73.0
	5730	5960 ± 20	104	192	187 ± 3	97.2
January 2, 2006	0	<LOQ		0	<LOQ	
	955	868 ± 20	90.9	32.0	29.5 ± 0.6	92.1
	1910	1730 ± 10	90.4	64.0	67.8 ± 0.3	106
	2870	2910 ± 150	102	96.0	97.2 ± 2.1	101
	3820	4200 ± 110	110	128	129 ± 2	101
	5730	6120 ± 20	107	192	195 ± 8	102
January 9, 2006	0	<LOQ		0	<LOQ	
	955	886 ± 30	92.7	32.0	32.0 ± 0.0	99.9
	1910	1830 ± 10	96.0	64.0	64.1 ± 1.1	100
	2870	3180 ± 60	111	96.0	98.3 ± 1.8	102
	3820	4270 ± 120	112	128	133 ± 1	104
	5730	6120 ± 20	108	192	201 ± 0	105
January 19, 2006	0	<LOQ		0	<LOQ	
	955	866 ± 9	90.7	32.0	33.3 ± 1.0	104
	1910	1780 ± 10	93.0	64.0	67.5 ± 1.1	106
	2870	2770 ± 60	96.8	96.0	97.4 ± 1.5	101
	3820	4020 ± 10	105	128	134 ± 1	105
	5730	5820 ± 10	102	192	199 ± 6	104
January 23, 2006	0	<LOQ		0	<LOQ	
	955	886 ± 42	92.8	32.0	33.9 ± 0.1	106
	1910	1840 ± 10	96.6	64.0	67.1 ± 0.6	105
	2870	3190 ± 70	111	96.0	104 ± 1	109
	3820	4280 ± 20	112	128	138 ± 2	108
	5730	6020 ± 260	105	192	206 ± 1	107

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
February 2, 2006	0	<LOQ		0	<LOQ	
	955	861 ± 70	90.2	32.0	34.1 ± 1.8	107
	1910	1880 ± 20	98.2	64.0	68.1 ± 0.3	106
	2870	2710 ± 210	94.4	96.0	105 ± 3	109
	3820	4120 ± 100	108	128	141 ± 1	110
	5730	5640 ± 160	98.4	192	212 ± 1	111
February 6, 2006	0	<LOQ		0	<LOQ	
	955	933 ± 25	97.7	32.0	32.1 ± 1.4	100
	1910	1930 ± 40	101	64.0	65.0 ± 0.4	102
	2870	2860 ± 20	99.7	96.0	96.9 ± 0.8	101
	3820	4160 ± 250	109	128	128 ± 3	99.9
	5730	6060 ± 220	106	192	184 ± 2	95.7
February 16, 2006	0	<LOQ		0	<LOQ	
	955	889 ± 15	93.1	32.0	33.8 ± 0.2	106
	1910	1900 ± 40	99.5	64.0	68.6 ± 2.0	107
	2870	2930 ± 110	102	96.0	102 ± 2	107
	3820	4000 ± 20	105	128	143 ± 2	112
	5730	5870 ± 160	102	192	209 ± 2	109
February 23, 2006	0	<LOQ		0	<LOQ	
	955	879 ± 27	92.1	32.0	35.8 ± 0.7	112
	1910	1860 ± 10	97.5	64.0	70.7 ± 1.1	111
	2870	2820 ± 180	98.6	96.0	106 ± 1	110
	3820	3890 ± 160	102	128	144 ± 1	112
	5730	5680 ± 40	99.2	192	208 ± 2	108
March 2, 2006	0	<LOQ		0	<LOQ	
	955	846 ± 13	88.6	32.0	35.4 ± 0.6	111
	1910	1840 ± 50	96.3	64.0	70.4 ± 0.9	110
	2870	2830 ± 40	98.8	96.0	110 ± 3	114
	3820	4100 ± 60	107	128	148 ± 1	115
	5730	5880 ± 170	103	192	216 ± 1	113
March 9, 2006	0	<LOQ		0	<LOQ	
	1020	788 ± 29	77.6	36.1	30.1 ± 0.6	83.4
	2030	1700 ± 30	83.7	72.1	63.6 ± 0.1	88.2
	3050	2640 ± 90	86.7	108	94.8 ± 0.4	87.8
	4060	3680 ± 40	90.5	144	124 ± 3	86.2
	6090	5520 ± 50	90.6	216	191 ± 1	88.3
March 16, 2006	0	<LOQ		0	<LOQ	
	1020	895 ± 17	88.2	36.1	31.6 ± 0.1	87.4
	2030	1770 ± 70	87.2	72.1	58.0 ± 0.4	80.4
	3050	2780 ± 10	91.3	108	93.9 ± 0.3	87.0
	4060	3810 ± 100	93.9	144	126 ± 2	87.3
	6090	5390 ± 110	88.5	216	186 ± 0	86.1
March 20, 2006	0	<LOQ		0	<LOQ	
	1020	869 ± 24	85.6	36.1	29.5 ± 0.1	81.6
	2030	1870 ± 0	92.0	72.1	60.6 ± 0.5	84.0
	3050	2830 ± 20	92.9	108	91.0 ± 1.7	84.3
	4060	3840 ± 10	94.6	144	121 ± 1	84.3
	6090	5710 ± 110	93.7	216	191 ± 1	88.5

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
March 27, 2006	0	<LOQ		0	<LOQ	
	1020	922 ± 19	90.8	36.1	30.4 ± 0.6	84.3
	2030	1860 ± 70	91.7	72.1	60.1 ± 1.5	83.4
	3050	2770 ± 10	90.8	108	93.3 ± 0.9	86.4
	4060	3710 ± 10	91.4	144	124 ± 1	86.1
	6090	5580 ± 50	91.6	216	191 ± 1	88.3
April 6, 2006	0	<LOQ		0	<LOQ	
	1020	847 ± 5	83.5	36.1	30.4 ± 0.3	84.1
	2030	1760 ± 30	86.7	72.1	61.3 ± 0.0	85.1
	3050	2680 ± 10	88.1	108	93.2 ± 0.1	86.3
	4060	3700 ± 20	91.1	144	127 ± 1	88.4
	6090	5450 ± 40	89.5	216	189 ± 1	87.7
April 10, 2006	0	<LOQ		0	<LOQ	
	1020	843 ± 0	83.1	36.1	31.4 ± 0.2	87.0
	2030	1790 ± 60	88.1	72.1	64.2 ± 2.4	89.0
	3050	2820 ± 20	92.6	108	96.5 ± 0.0	89.4
	4060	3730 ± 50	92.0	144	127 ± 1	88.0
	6090	5570 ± 130	91.4	216	188 ± 4	86.9
April 13, 2006	0	<LOQ		0	<LOQ	
	1020	987 ± 15	97.2	36.1	36.4 ± 0.8	101
	2030	1740 ± 10	85.7	72.1	62.4 ± 0.7	86.5
	3050	2680 ± 10	88.0	108	94.9 ± 0.2	87.9
	4060	3590 ± 20	88.4	144	127 ± 2	88.5
	6090	5310 ± 50	87.1	216	187 ± 1	86.4
April 17, 2006	0	<LOQ		0	<LOQ	
	1020	824 ± 2	81.2	36.1	33.2 ± 0.4	92.1
	2030	1780 ± 20	87.5	72.1	66.0 ± 1.1	91.5
	3050	2760 ± 50	90.7	108	99.2 ± 2.3	91.9
	4060	3780 ± 40	93.1	144	135 ± 1	93.8
	6090	5600 ± 10	91.9	216	205 ± 4	95.0
April 27, 2006	0	<LOQ		0	<LOQ	
	1020	874 ± 12	86.1	36.1	33.0 ± 0.7	91.4
	2030	1800 ± 0	88.5	72.1	68.2 ± 2.1	94.5
	3050	2770 ± 50	91.1	108	99.3 ± 0.7	91.9
	4060	3660 ± 50	90.2	144	134 ± 1	92.9
	6090	5600 ± 430	91.9	216	198 ± 2	91.5
May 1, 2006	0	<LOQ		0	<LOQ	
	1020	873 ± 27	86.0	36.1	33.0 ± 0.0	91.4
	2030	1840 ± 20	90.8	72.1	66.7 ± 1.7	92.6
	3050	2750 ± 10	90.4	108	101 ± 1	93.1
	4060	3610 ± 50	88.9	144	134 ± 2	92.8
	6090	5460 ± 30	89.7	216	199 ± 0	92.1
May 8, 2006	0	<LOQ		0	<LOQ	
	1020	867 ± 12	85.4	36.1	30.2 ± 0.9	83.7
	2030	1850 ± 40	91.0	72.1	62.7 ± 0.2	87.0
	3050	2700 ± 50	88.5	108	97.2 ± 1.1	90.0
	4060	3660 ± 50	90.2	144	131 ± 2	90.7
	6090	5490 ± 30	90.2	216	194 ± 1	89.9

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
May 18, 2006	0	<LOQ		0	<LOQ	
	1020	840 ± 7	82.8	36.1	33.3 ± 0.6	92.3
	2030	1790 ± 40	88.0	72.1	69.3 ± 0.5	96.1
	3050	2780 ± 0	91.2	108	103 ± 1	95.4
	4060	3680 ± 20	90.7	144	139 ± 1	96.6
	6090	5550 ± 30	91.1	216	207 ± 1	95.7
May 22, 2006	0	<LOQ		0	<LOQ	
	1020	879 ± 41	86.6	36.1	31.4 ± 0.2	87.0
	2030	1740 ± 40	85.9	72.1	65.0 ± 1.2	90.1
	3050	2620 ± 20	86.0	108	97.2 ± 0.6	90.0
	4060	3590 ± 20	88.4	144	130 ± 1	90.1
	6090	5230 ± 0	86.0	216	196 ± 1	90.5
May 29, 2006	0	<LOQ		0	<LOQ	
	1020	849 ± 5	83.7	36.1	28.5 ± 0.3	79.1
	2030	1820 ± 70	89.9	72.1	56.9 ± 0.9	78.9
	3050	2820 ± 30	92.7	108	86.0 ± 0.2	79.6
	4060	3780 ± 10	93.2	144	118 ± 0	82.0
	6090	5550 ± 110	91.2	216	177 ± 0	81.8
June 8, 2006	0	<LOQ		0	<LOQ	
	1020	844 ± 35	83.1	36.1	31.1 ± 0.7	86.1
	2030	1870 ± 140	91.9	72.1	63.6 ± 0.8	88.2
	3050	2810 ± 40	92.3	108	90.2 ± 0.0	83.5
	4060	3680 ± 50	90.7	144	123 ± 2	85.4
	6090	5400 ± 80	88.7	216	187 ± 1	86.4
June 12, 2006	0	<LOQ		0	<LOQ	
	1020	869 ± 12	85.6	36.1	31.9 ± 0.3	88.4
	2030	1810 ± 0	89.2	72.1	64.9 ± 2.4	90.0
	3050	2760 ± 30	90.7	108	97.9 ± 1.5	90.7
	4060	3830 ± 20	94.3	144	131 ± 1	90.8
	6090	5410 ± 190	88.9	216	192 ± 2	88.8
June 19, 2006	0	<LOQ		0	<LOQ	
	1020	841 ± 19	82.9	36.1	29.4 ± 0.4	81.4
	2030	1810 ± 40	89.4	72.1	57.0 ± 2.2	79.1
	3050	2780 ± 30	91.4	108	87.6 ± 2.1	81.1
	4060	3630 ± 10	89.3	144	120 ± 2	83.0
	6090	5570 ± 240	91.5	216	183 ± 1	84.6
June 26, 2006	0	<LOQ		0	<LOQ	
	1020	879 ± 15	86.6	36.1	29.8 ± 0.3	82.5
	2030	1760 ± 60	86.7	72.1	61.8 ± 0.3	85.7
	3050	2740 ± 20	90.0	108	89.8 ± 0.3	83.1
	4060	3700 ± 140	91.1	144	118 ± 1	81.8
	6090	5310 ± 150	87.2	216	188 ± 2	86.9
July 6, 2006	0	<LOQ		0	<LOQ	
	1020	837 ± 25	82.4	36.1	29.4 ± 0.2	81.4
	2030	1770 ± 0	87.0	72.1	61.8 ± 1.8	85.7
	3050	2690 ± 40	88.3	108	87.9 ± 0.9	81.4
	4060	3620 ± 70	89.1	144	117 ± 0	80.9
	6090	5470 ± 150	89.8	216	180 ± 1	83.5

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
July 13, 2006	0	<LOQ		0	<LOQ	
	1020	843 ± 10	83.0	36.1	29.2 ± 0.2	80.8
	2030	1780 ± 0	87.7	72.1	61.6 ± 0.1	85.5
	3050	2730 ± 80	89.6	108	89.8 ± 0.4	83.1
	4060	3650 ± 40	90.0	144	119 ± 1	82.4
	6090	5670 ± 70	93.0	216	183 ± 1	84.7
July 20, 2006	0	<LOQ		0	<LOQ	
	1020	820 ± 40	80.7	36.1	29.2 ± 0.2	80.8
	2030	1750 ± 0	86.4	72.1	59.6 ± 0.3	82.7
	3050	2510 ± 20	82.5	108	87.2 ± 0.5	80.7
	4060	3380 ± 40	83.2	144	118 ± 1	82.0
	6090	4980 ± 40	81.9	216	177 ± 0	81.9
July 27, 2006	0	<LOQ		0	<LOQ	
	1020	860 ± 40	84.4	36.1	34.3 ± 0.2	95.0
	2030	1810 ± 40	89.1	72.1	66.8 ± 0.3	92.6
	3050	2750 ± 70	90.4	108	97.2 ± 0.8	90.0
	4060	3800 ± 80	93.6	144	134 ± 1	92.8
	6090	5410 ± 80	88.9	216	198 ± 1	91.6
August 3, 2006	0	<LOQ		0	<LOQ	
	930	790 ± 20	84.7	28.9	32.3 ± 0.6	112
	1860	1700 ± 10	91.4	57.8	65.1 ± 2.0	113
	2790	2640 ± 0	94.6	86.7	95.8 ± 0.0	110
	3720	3550 ± 50	95.4	116	127 ± 1	110
	5580	5690 ± 310	102	173	195 ± 1	112
August 10, 2006	0	<LOQ		0	<LOQ	
	930	848 ± 33	91.2	28.9	34.3 ± 0.8	119
	1860	1830 ± 40	98.4	57.8	72.0 ± 0.7	125
	2790	2730 ± 20	98.0	86.7	104 ± 0.5	120
	3720	3750 ± 20	101	116	139 ± 2	120
	5580	5690 ± 250	102	173	213 ± 2	123
August 17, 2006	0	<LOQ		0	<LOQ	
	930	851 ± 2	91.5	28.9	32.4 ± 0.9	112
	1860	1790 ± 0	96.1	57.8	65.7 ± 0.4	114
	2790	2760 ± 80	99.0	86.7	95.8 ± 0.7	111
	3720	3670 ± 30	98.6	116	126 ± 1	109
	5580	5450 ± 30	97.7	173	198 ± 0	114
August 21, 2006	0	<LOQ		0	<LOQ	
	930	873 ± 31	93.9	28.9	33.3 ± 0.3	115
	1860	1910 ± 120	103	57.8	66.3 ± 0.7	115
	2790	2760 ± 30	99.0	86.7	96.2 ± 1.5	111
	3720	3720 ± 10	100	116	130 ± 1	113
	5580	5690 ± 150	102	173	199 ± 0	115
August 31, 2006	0	<LOQ		0	<LOQ	
	930	890 ± 7	95.7	28.9	28.2 ± 0.5	97.7
	1860	1850 ± 50	99.7	57.8	62.8 ± 0.6	109
	2790	2780 ± 40	99.5	86.7	88.9 ± 0.5	103
	3720	3750 ± 100	101	116	124 ± 1	107
	5580	5480 ± 170	98.2	173	181 ± 2	104

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
September 7, 2006	0	<LOQ		0	<LOQ	
	930	857 ± 29	92.2	28.9	30.5 ± 0.4	106
	1860	1830 ± 60	98.3	57.8	59.1 ± 1.3	102
	2790	2740 ± 10	98.2	86.7	86.3 ± 0.6	99.6
	3720	3690 ± 80	99.2	116	115 ± 0	99.4
	5580	5490 ± 140	98.4	173	166 ± 4	95.9
September 14, 2006	0	<LOQ		0	<LOQ	
	930	850 ± 7	91.4	28.9	29.5 ± 0.0	102
	1860	1730 ± 10	93.0	57.8	59.1 ± 0.7	102
	2790	2660 ± 10	95.3	86.7	87.1 ± 0.2	100
	3720	3660 ± 130	98.3	116	116 ± 0	101
	5580	5600 ± 230	100	173	179 ± 2	103
September 18, 2006	0	<LOQ		0	<LOQ	
	930	846 ± 24	90.9	28.9	30.4 ± 0.3	105
	1860	1770 ± 60	94.9	57.8	58.6 ± 0.7	101
	2790	2730 ± 30	98.0	86.7	87.2 ± 0.6	101
	3720	3710 ± 20	99.7	116	114 ± 2	99.0
	5580	5550 ± 130	99.5	173	171 ± 0	98.7
September 28, 2006	0	<LOQ		0	<LOQ	
	930	862 ± 21	92.7	28.9	30.9 ± 0.3	107
	1860	1800 ± 0	96.5	57.8	62.1 ± 0.4	107
	2790	2700 ± 20	96.8	86.7	89.3 ± 1.1	103
	3720	3620 ± 20	97.3	116	117 ± 4	101
	5580	5650 ± 130	101	173	191 ± 1	110
October 5, 2006	0	<LOQ		0	<LOQ	
	930	888 ± 10	95.5	28.9	29.7 ± 0.7	103
	1860	1820 ± 70	98.0	57.8	60.8 ± 0.6	105
	2790	2710 ± 20	97.0	86.7	85.0 ± 2.2	98.1
	3720	3810 ± 60	102	116	115 ± 0	99.5
	5580	5480 ± 140	98.3	173	199 ± 3	115
October 12, 2006	0	<LOQ		0	<LOQ	
	930	849 ± 35	91.3	28.9	29.5 ± 0.3	102
	1860	1710 ± 30	91.9	57.8	57.9 ± 0.1	100
	2790	2690 ± 0	96.4	86.7	83.7 ± 2.9	96.5
	3720	3700 ± 70	99.6	116	117 ± 0	101
	5580	6000 ± 80	108	173	197 ± 2	114
October 16, 2006	0	<LOQ		0	<LOQ	
	930	855 ± 37	91.9	28.9	32.4 ± 0.3	112
	1860	1790 ± 0	96.1	57.8	64.6 ± 1.0	112
	2790	2850 ± 30	102	86.7	94.1 ± 0.5	109
	3720	3790 ± 30	102	116	127 ± 2	110
	5580	5500 ± 0	98.5	173	202 ± 1	117
October 23, 2006	0	<LOQ		0	<LOQ	
	930	829 ± 5	89.1	28.9	32.3 ± 0.1	112
	1860	1780 ± 10	95.8	57.8	64.8 ± 0.0	112
	2790	2680 ± 60	96.0	86.7	92.2 ± 1.0	106
	3720	3660 ± 50	98.3	116	124 ± 1	107
	5580	5380 ± 60	96.4	173	202 ± 1	116

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
October 30, 2006	0	<LOQ		0	<LOQ	
	930	830 ± 21	89.3	28.9	30.3 ± 0.0	105
	1860	1720 ± 10	92.7	57.8	61.4 ± 0.7	106
	2790	2620 ± 90	93.9	86.7	89.3 ± 1.0	103
	3720	3520 ± 50	94.7	116	118 ± 2	102
	5580	5380 ± 70	96.4	173	179 ± 0	103
November 9, 2006	0	<LOQ		0	<LOQ	
	930	831 ± 14	89.4	28.9	29.9 ± 0.2	103
	1860	1810 ± 20	97.2	57.8	59.6 ± 0.2	103
	2790	2630 ± 20	94.4	86.7	84.7 ± 1.0	97.7
	3720	3330 ± 30	89.6	116	108 ± 0	93.7
	5580	5350 ± 80	95.9	173	174 ± 3	101
November 16, 2006	0	<LOQ		0	<LOQ	
	930	828 ± 12	89.0	28.9	30.7 ± 0.2	106
	1860	1750 ± 0	94.3	57.8	58.9 ± 0.4	102
	2790	2710 ± 20	97.2	86.7	87.7 ± 1.2	101
	3720	3670 ± 90	98.7	116	122 ± 3	105
	5580	5410 ± 20	96.9	173	182 ± 1	105
November 20, 2006	0	<LOQ		0	<LOQ	
	930	855 ± 29	92.0	28.9	32.2 ± 1.8	111
	1860	1770 ± 100	95.2	57.8	62.4 ± 0.4	108
	2790	2790 ± 130	100	86.7	92.4 ± 1.0	107
	3720	3720 ± 10	100	116	124 ± 0	107
	5580	5400 ± 230	96.7	173	190 ± 2	109
November 30, 2006	0	<LOQ		0	<LOQ	
	930	865 ± 22	93.0	28.9	32.6 ± 0.6	113
	1860	1880 ± 20	101	57.8	67.9 ± 0.9	117
	2790	2860 ± 80	103	86.7	96.5 ± 1.2	111
	3720	3890 ± 40	104	116	130 ± 1	112
	5580	5610 ± 60	101	173	193 ± 1	111
December 7, 2006	0	<LOQ		0	<LOQ	
	930	866 ± 36	93.2	28.9	30.4 ± 0.4	105
	1860	1820 ± 40	98.1	57.8	60.6 ± 0.2	105
	2790	2900 ± 90	104	86.7	93.1 ± 1.2	107
	3720	3880 ± 60	104	116	123 ± 1	106
	5580	5580 ± 70	99.9	173	188 ± 2	108
December 11, 2006	0	<LOQ		0	<LOQ	
	930	877 ± 19	94.3	28.9	32.6 ± 0.3	113
	1860	1820 ± 50	97.8	57.8	66.0 ± 0.2	114
	2790	2780 ± 30	99.6	86.7	97.0 ± 0.2	112
	3720	3670 ± 50	98.7	116	128 ± 1	111
	5580	5470 ± 210	98.0	173	190 ± 1	110
December 18, 2006	0	<LOQ		0	<LOQ	
	930	847 ± 12	91.1	28.9	31.5 ± 0.3	109
	1860	1830 ± 50	98.5	57.8	63.9 ± 1.0	111
	2790	2720 ± 90	97.4	86.7	92.3 ± 0.7	106
	3720	3620 ± 60	97.3	116	124 ± 2	107
	5580	5930 ± 200	106	173	189 ± 1	109

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
December 28, 2006	0	<LOQ		0	<LOQ	
	965	853 ± 7	88.4	34.5	31.5 ± 0.1	91.5
	1930	1780 ± 40	92.4	68.9	64.5 ± 1.9	93.6
	2900	2730 ± 50	94.3	103	94.2 ± 0.2	91.1
	3860	3650 ± 40	94.5	138	128 ± 1	92.7
	5790	5460 ± 100	94.3	207	192 ± 2	93.0
January 4, 2007	0	<LOQ		0	<LOQ	
	965	841 ± 7	87.1	34.5	29.6 ± 0.0	86.0
	1930	1850 ± 30	95.9	68.9	62.8 ± 0.2	91.2
	2900	2720 ± 20	94.0	103	86.9 ± 0.2	84.1
	3860	3720 ± 80	96.5	138	115 ± 1	83.2
	5790	5460 ± 170	94.3	207	174 ± 3	84.0
January 11, 2007	0	<LOQ		0	<LOQ	
	965	472 ± 19	48.9	34.5	16.1 ± 0.3	46.8
	1930	1040 ± 0	54.0	68.9	32.9 ± 0.5	47.7
	2900	1640 ± 20	56.5	103	49.9 ± 0.1	48.3
	3860	2210 ± 30	57.1	138	67.4 ± 1.1	48.9
	5790	3210 ± 40	55.5	207	108 ± 2	52.0
January 18, 2007	0	<LOQ		0	<LOQ	
	965	1090 ± 50	113	34.5	37.4 ± 0.8	109
	1930	1660 ± 60	86.0	68.9	54.9 ± 0.3	79.7
	2900	2730 ± 80	94.3	103	90.4 ± 2.3	87.5
	3860	3630 ± 10	94.1	138	122 ± 1	88.6
	5790	5430 ± 30	93.8	207	183 ± 1	88.6
January 22, 2007	0	<LOQ		0	<LOQ	
	965	876 ± 19	90.7	34.5	29.8 ± 0.6	86.5
	1930	1860 ± 0	96.2	68.9	61.4 ± 0.2	89.1
	2900	2830 ± 30	97.8	103	93.5 ± 0.3	90.5
	3860	3740 ± 20	97.0	138	125 ± 0	90.4
	5790	5870 ± 100	102	207	190 ± 3	91.8
January 29, 2007	0	<LOQ		0	<LOQ	
	965	899 ± 3	93.2	34.5	30.4 ± 0.2	88.3
	1930	1840 ± 30	95.2	68.9	62.4 ± 0.3	90.6
	2900	2830 ± 70	97.6	103	94.6 ± 0.3	91.6
	3860	3830 ± 130	99.3	138	128 ± 1	92.7
	5790	5550 ± 130	95.8	207	192 ± 1	92.9
February 8, 2007	0	<LOQ		0	<LOQ	
	965	866 ± 29	89.7	34.5	31.1 ± 0.1	90.3
	1930	1870 ± 20	96.9	68.9	61.6 ± 0.0	89.5
	2900	2730 ± 70	94.2	103	95.9 ± 0.0	92.8
	3860	3900 ± 110	101	138	130 ± 1	94.7
	5790	5770 ± 0	99.6	207	195 ± 2	94.5
February 15, 2007	0	<LOQ		0	<LOQ	
	965	835 ± 34	86.6	34.5	31.6 ± 0.8	91.6
	1930	1840 ± 60	95.6	68.9	63.8 ± 0.9	92.6
	2900	2770 ± 0	95.7	103	90.5 ± 1.0	87.6
	3860	3640 ± 10	94.4	138	119 ± 1	86.6
	5790	5440 ± 40	94.0	207	180 ± 1	87.1

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
February 22, 2007	0	<LOQ		0	<LOQ	
	965	842 ± 34	87.3	34.5	32.0 ± 0.4	92.9
	1930	1740 ± 20	90.1	68.9	64.0 ± 0.2	92.8
	2900	2640 ± 0	91.1	103	89.9 ± 0.5	86.9
	3860	3610 ± 50	93.6	138	121 ± 3	88.2
	5790	5200 ± 0	89.8	207	183 ± 1	88.3
March 1, 2007	0	<LOQ		0	<LOQ	
	965	898 ± 19	93.1	34.5	34.6 ± 0.6	100
	1930	1830 ± 0	94.7	68.9	69.8 ± 1.0	101
	2900	2900 ± 50	100	103	97.0 ± 0.4	93.8
	3860	3860 ± 60	100	138	129 ± 1	93.5
	5790	5670 ± 440	97.9	207	194 ± 1	93.6
March 5, 2007	0	<LOQ		0	<LOQ	
	965	831 ± 17	86.1	34.5	32.4 ± 0.4	94.0
	1930	1800 ± 90	93.4	68.9	65.7 ± 0.5	95.3
	2900	2860 ± 20	98.8	103	92.0 ± 0.0	89.0
	3860	3820 ± 10	99.1	138	122 ± 2	88.5
	5790	5890 ± 70	102	207	185 ± 0	89.3
March 15, 2007	0	<LOQ		0	<LOQ	
	965	909 ± 28	94.2	34.5	30.3 ± 0.5	87.9
	1930	1780 ± 20	92.4	68.9	59.9 ± 0.3	86.9
	2900	2830 ± 110	97.7	103	85.3 ± 0.5	82.5
	3860	3810 ± 20	98.6	138	116 ± 2	84.5
	5790	5390 ± 30	93.0	207	170 ± 1	82.1
March 22, 2007	0	<LOQ		0	<LOQ	
	965	874 ± 35	90.5	34.5	32.1 ± 0.0	93.2
	1930	1760 ± 10	91.3	68.9	64.8 ± 0.2	94.0
	2900	2660 ± 10	91.8	103	94.9 ± 0.2	91.8
	3860	3630 ± 0	94.2	138	127 ± 1	92.5
	5790	5440 ± 150	94.0	207	196 ± 2	94.6
March 29, 2007	0	<LOQ		0	<LOQ	
	965	875 ± 43	90.7	34.5	31.7 ± 0.6	92.0
	1930	1870 ± 60	96.8	68.9	64.6 ± 0.3	93.7
	2900	2800 ± 40	96.8	103	95.1 ± 0.1	92.0
	3860	3830 ± 30	99.1	138	129 ± 1	93.3
	5790	5360 ± 50	92.5	207	191 ± 2	92.1
April 2, 2007	0	<LOQ		0	<LOQ	
	965	875 ± 43	93.1	34.5	31.7 ± 0.5	91.9
	1930	1870 ± 60	97.8	68.9	64.7 ± 1.9	93.9
	2900	2800 ± 40	98.6	103	93.2 ± 2.3	90.2
	3860	3830 ± 30	100	138	125 ± 1	90.4
	5790	5360 ± 50	97.3	207	192 ± 1	92.7
April 12, 2007	0	<LOQ		0	<LOQ	
	965	854 ± 0	88.5	34.5	32.0 ± 0.1	92.8
	1,930	1,820 ± 30	94.1	68.9	65.7 ± 0.4	95.3
	2,900	2,890 ± 70	99.7	103	96.0 ± 0.8	92.9
	3,860	3,840 ± 30	99.5	138	128 ± 1	93.2
	5,790	5,620 ± 100	97.0	207	194 ± 1	93.9

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Date Prepared and Sampled	Malic acid			Aloin A		
	Target (ppm)	Determined (ppm)	% Target	Target (ppm)	Determined (ppm)	% Target
April 16, 2007	0	<LOQ		0	<LOQ	
	965	864 ± 13	89.5	34.5	32.2 ± 0.2	93.5
	1,930	1,840 ± 70	95.1	68.9	62.5 ± 0.0	90.7
	2,900	2,990 ± 130	103	103	90.3 ± 0.3	87.4
	3,860	4,030 ± 140	104	138	124 ± 5	89.8
	5,790	6,150 ± 540	106	207	205 ± 2	99.3
April 23, 2007	0	<LOQ		0	<LOQ	
	965	841 ± 25	87.2	34.5	33.6 ± 0.2	97.5
	1,930	1,860 ± 210	96.1	68.9	64.6 ± 1.8	93.8
	2,900	2,640 ± 40	91.3	103	97.0 ± 0.0	93.8
	3,860	3,830 ± 10	99.2	138	130 ± 1	94.4
	5,790	5,620 ± 110	97.1	207	200 ± 3	96.7
April 30, 2007	0	<LOQ		0	<LOQ	
	965	772 ± 44	80.0	34.5	32.8 ± 2.0	95.3
	1,930	1,770 ± 80	91.8	68.9	68.3 ± 0.4	99.2
	2,900	2,740 ± 110	94.7	103	96.8 ± 0.2	93.7
	3,860	3,640 ± 90	94.2	138	129 ± 1	93.3
	5,790	5,870 ± 560	102	207	198 ± 2	95.7
May 10, 2007	0	<LOQ		0	<LOQ	
	965	902 ± 12	93.4	34.5	31.2 ± 2.8	90.6
	1,930	1,900 ± 20	98.3	68.9	66.5 ± 0.3	96.5
	2,900	2,540 ± 20	87.9	103	85.8 ± 0.6	83.0
	3,860	3,160 ± 40	81.8	138	103 ± 0	75.0
	5,790	5,630 ± 120	97.2	207	195 ± 2	94.4
May 14, 2007	0	<LOQ		0	<LOQ	
	965	1120 ± 0	116	34.5	41.2 ± 2.1	120
	1,930	1,860 ± 20	96.6	68.9	67.5 ± 0.1	98.0
	2,900	2,900 ± 60	100	103	101 ± 1	97.5
	3,860	4,180 ± 30	108	138	144 ± 2	105
	5,790	5,690 ± 90	98.2	207	223 ± 6	108
May 21, 2007	0	<LOQ		0	<LOQ	
	965	910 ± 0	94.3	34.5	34.9 ± 0.2	101
	1,930	1,930 ± 30	99.8	68.9	68.3[2]	99.1
	2,900	2,960 ± 30	102	103	104 ± 2	100
	3,860	4,090 ± 370	106	138	135 ± 2	97.6
	5,790	5,770 ± 10	99.6	207	210 ± 2	102

[a] Lot # WLN-5001A was utilized from 4/25/05 to 10/13/05; lot # WLN-5001B was utilized from 10/17/05 to 3/2/06; lot # WLN-6001A was utilized from 3/6/06 to 7/27/06; lot # WLN-6001B was utilized from 7/31/06 to 12/21/06; lot # WLN-6001C was utilized from 12/25/06 through the end of study.
[b] The limits of quantitation were estimated to be 100 ppm malic acid and 0.1 ppm aloin A.
[c] Formulations sampled July 8, 2005 were prepared on July 7, 2005.

TABLE H5
Results of Analyses of Animal Room Samples in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

		Malic acid			Aloin A		
Date Prepared	**Date Sampled**	**Target (ppm)**	**Result (ppm)**	**% Target**	**Target (ppm)**	**Result (ppm)**	**% Target**
April 17, 2006	April 17, 2006	0	<LOQ[a]		0	<LOQ	
		0	<LOQ		0	<LOQ	
		1,020	907 ± 5	89.3	36.1	28.0 ± 0.0	77.7
		2,030	1,690 ± 40	83.1	72.1	44.0 ± 0.9	61.1
		2,030	1,020 ± 30	50.0	72.1	43.9 ± 0.9	60.9
		3,050	2,840 ± 50	93.4	108	67.9 ± 0.5	62.8
		4,060	546 ± 12	13.4	144	87.3 ± 3.1	60.6
		6,090	5,580 ± 140	89.9	216	121 ± 1	56.0
July 17, 2006	July 17, 2006	0	<LOQ		0	<LOQ	
		0	<LOQ		0	<LOQ	
		1,020	137 ± 10	13.5	36.1	24.8 ± 0.3	68.6
		2,030	1,390 ± 0	68.4	72.1	46.8 ± 0.2	64.9
		2,030	<LOQ[b]	0	72.1	46.1 ± 0.4	64.0
		3,050	1,370 ± 10	67.6	108	71.1 ± 0.7	65.8
		4,060	<LOQ[b,c]	0	144	83.9 ± 2.5	58.2
		6,090	<LOQ[b]	0	216	125 ± 3	57.9
October 16, 2006	October 16, 2006	0	<LOQ		0	<LOQ	
		930	629 ± 2	67.7	28.9	24.9 ± 0.2	86.2
		1,860	1,700 ± 40	91.3	57.8	47.2 ± 0.6	81.7
		2,790	2,740 ± 20	98.3	86.7	69.3 ± 0.7	79.9
October 23, 2006	October 23, 2006	0	<LOQ		0	<LOQ	
		1,860	1,370 ± 20	73.8	57.8	48.7 ± 0.3	84.2
		3,720	3,100 ± 60	83.4	116	89.9 ± 1.4	77.8
		5,580	5,160 ± 110	92.4	173	144 ± 1	83.3
January 15, 2007	January 17, 2007	0	<LOQ		0	<LOQ	
		965	<LOQ	---	34.5	25.4 ± 0.8	73.8
		1,930	1,340 ± 30	69.5	68.9	49.7 ± 1.1	72.1
		2,900	2,010 ± 120	69.3	103	70.4 ± 0.8	68.1
January 22, 2007	January 22, 2007	0	<LOQ		0	<LOQ	
		1930	1,430 ± 10	74.0	68.9	42.3 ± 0.4	61.4
		3,860	3,590 ± 20	93.0	138	86.3 ± 0.8	62.6
		5,790	<LOQ		207	138 ± 3	67.0
March 29, 2007	April 1, 2007	0	<LOQ		0	<LOQ	
		965	486 ± 12	50.3	34.5	24.1 ± 0.1	69.9
		1,930	648 ± 106	33.6	68.9	45.1 ± 2.4	65.4
		2,900	1,600 ± 130	55.3	103	65.8 ± 0.7	63.7
April 23, 2007	April 23, 2007	0	<LOQ		0	<LOQ	
		1930	1,360 ± 70	70.5	68.9	48.9 ± 1.2	71.0
		3,860	3,550 ± 20	92.0	138	96.9 ± 0.9	70.3
		5,790	5,430 ± 90	93.9	207	125 ± 0	60.5

[a] The limits of quantitation were estimated to be 100 ppm malic acid and 0.1 ppm aloin A.
[b] These results confirmed by separate analyses.
[c] Absence of malic acid was confirmed by standard addition.

TABLE H6
Results for Glycosyl Linkage Analyses
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

Glycosyl Residue	Percent Present
WLN05001A	
Terminal Arabinopyranose	2.4
Terminal Xylopyranose	1.7
Terminal Mannopyranose	3.0
Terminal Glucopyranose	15.3
Terminal Galactopyranose	0.7
3 Linked Glucopyranose	6.6
4 Linked Mannopyranose	37.2
6 Linked Glucopyranose	4.3
4 Linked Glucopyranose	13.5
3,4 Linked Mannopyranose	1.6
3,4 Linked Glucopyranose	8.0
4,6 Linked Mannopyranose	2.2
4,6 Linked Glucopyranose	2.1
3,4,6 Linked Glucopyranose	1.4
WLN05001B	
Terminal Arabinopyranose	5.3
Terminal Xylopyranose	2.2
Terminal Mannopyranose	3.2
Terminal Glucopyranose	8.8
3 Linked Glucopyranose	4.2
4 Linked Mannopyranose	43.5
6 Linked Glucopyranose	4.3
4 Linked Glucopyranose	9.4
3,4 Linked Mannopyranose	4.2
3,4 Linked Glucopyranose	6.4
4,6 Linked Mannopyranose	5.2
4,6 Linked Glucopyranose	1.7
3,4,6 Linked Glucopyranose	1.3
WLN06001A	
Terminal Arabinopyranose	2.0
Terminal Xylopyranose	2.6
Terminal Mannopyranose	3.1
Terminal Glucopyranose	13.4
3 Linked Glucopyranose	5.6
4 Linked Mannopyranose	37.5
6 Linked Glucopyranose	4.6
4 Linked Glucopyranose	13.0
3,4 Linked Mannopyranose	2.0
3,4 Linked Glucopyranose	7.5
4,6 Linked Mannopyranose	4.4
4,6 Linked Glucopyranose	2.6
3,4,6 Linked Glucopyranose	1.7
WLN06001B	
Terminal Arabinopyranose	3.6
Terminal Xylopyranose	3.3
Terminal Mannopyranose	2.6
Terminal Glucopyranose	13.2
3 Linked Glucopyranose	5.6
4 Linked Mannopyranose	40.1
6 Linked Glucopyranose	4.7
4 Linked Glucopyranose	11.4
3,4 Linked Mannopyranose	4.2
3,4 Linked Glucopyranose	7.5
4,6 Linked Mannopyranose	3.8

TABLE H6
Results for Glycosyl Linkage Analyses
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract (continued)

Glycosyl Residue	Percent Present
WLN06001C	
Terminal Arabinopyranose	0.9
Terminal Xylopyranose	1.8
Terminal Mannopyranose	3.0
Terminal Glucopyranose	21.0
Terminal Galactopyranose	1.7
3 Linked Glucopyranose	5.3
4 Linked Mannopyranose	33.9
6 Linked Glucopyranose	4.5
4 Linked Glucopyranose	13.8
3,4 Linked Mannopyranose	1.2
3,4 Linked Glucopyranose	7.5
4,6 Linked Mannopyranose	2.2
4,6 Linked Glucopyranose	2.1
3,4,6 Linked Glucopyranose	1.1

TABLE H7
Results for Average Molecular Weight Analysis of Polysaccharides
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

Sample Designation	Date Reported	Molecular Weight (kDa)	%RSD
WLN-05001A	8/02/05	61.3 ± 1.8	3.0
WLN-05001B	9/28/05	52.1 ± 2.7	5.1
WLN-06001A	10/30/07	61.3 ± 4.7	7.7
WLN-06001B	10/30/07	56.9 ± 6.3	11
WLN-06001C	10/30/07	78.3 ± 0.6	0.8

APPENDIX I
FEED CONSUMPTION IN THE 14-DAY AND 13-WEEK DRINKING WATER STUDIES OF ALOE VERA EXTRACTS

TABLE I1
Feed Consumption of Rats in the 14-Day Drinking Water Study of Aloe vera Extracts

Aloe vera Extract and Concentration (%)	Mean Feed Consumption[a]		
	Week 0	Week 1	Week 2
Male			
Gel			
0	16.85 ± 0.98	14.71 ± 0.78	14.81 ± 1.54
0.5	16.59 ± 0.98	14.72 ± 0.78	16.28 ± 1.54
1	16.82 ± 0.98	14.81 ± 0.78	14.25 ± 1.54
1.5	17.30 ± 0.98	14.79 ± 0.78	14.66 ± 1.54
2	17.51 ± 0.98	16.23 ± 0.78	14.67 ± 1.54
3	16.65 ± 0.98	15.48 ± 0.78	14.25 ± 1.54
Decolorized Whole Leaf			
0	16.03 ± 0.78	15.83 ± 0.60	15.66 ± 1.60
0.5	15.80 ± 0.78	15.03 ± 0.60	15.67 ± 1.60
1	15.03 ± 0.78	15.24 ± 0.60	14.17 ± 1.60
1.5	16.24 ± 0.78	14.80 ± 0.60	14.92 ± 1.60
2	16.12 ± 0.78	15.21± 0.60	15.20 ± 1.60
3	15.55 ± 0.78	14.45 ± 0.60	14.90 ± 1.60
Whole Leaf			
0	16.02 ± 0.75	14.90 ± 0.66*	14.80 ± 1.59
0.5	17.24 ± 0.75	15.05 ± 0.66	14.82 ± 1.59
1	17.20 ± 0.75	15.63 ± 0.66	14.10 ± 1.59
1.5	16.73 ± 0.75	13.34 ± 0.66	14.05 ± 1.59
2	16.72 ± 0.75	14.52 ± 0.66	13.01 ± 1.59
3	15.09 ± 0.75	10.36 ± 0.66*	11.14 ± 1.59*
Female			
Gel			
0	13.82 ± 1.66	10.97 ± 1.47	10.64 ± 0.83
0.5	14.96 ± 1.66	13.49 ± 1.47	10.81 ± 0.83
1	14.91 ± 1.66	15.25 ± 1.47	11.63 ± 0.83
1.5	14.62 ± 1.66	11.16 ± 1.47	10.54 ± 0.83
2	14.18 ± 1.66	12.69 ± 1.47	10.26 ± 0.83
3	14.32 ± 1.66	12.89 ± 1.47	10.92 ± 0.83
Decolorized Whole Leaf			
0	13.86 ± 0.80	12.36 ± 0.44	11.33 ± 0.75
0.5	13.05 ± 0.80	11.71 ± 0.44	11.51 ± 0.75
1	14.04 ± 0.80	11.79 ± 0.44	10.76 ± 0.75
1.5	14.72 ± 0.80	11.77 ± 0.44	10.76 ± 0.75
2	13.42 ± 0.80	11.88 ± 0.44	10.76 ± 0.75
3	13.48 ± 0.80	11.88 ± 0.44	11.31 ± 0.75
Whole Leaf			
0	14.25 ± 0.74	11.82 ± 0.96	9.97 ± 1.02*
0.5	14.08 ± 0.74	11.52 ± 0.96	11.30 ± 1.02
1	12.57 ± 0.74	11.43 ± 0.96	10.40 ± 1.02
1.5	13.40 ± 0.74	10.56 ± 0.96	8.99 ± 1.02
2	13.77 ± 0.74	10.52 ± 0.96	7.87 ± 1.02
3	13.76 ± 0.74	9.63 ± 0.96	6.81 ± 1.02

[a] Feed consumption is expressed as grams per animal per day.
* Signifies values that are significantly different ($P \leq 0.05$) from control group by Dunnett's test and significant linear dose trend ($P \leq 0.05$) effects based on contrast comparisons for control group.

TABLE I2
Feed Consumption of Rats in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract

Concentration (%)	Mean Feed Consumption[a]			
	Week 0	Week 4	Week 8	Week 13
Subchronic Study				
Male				
0	17.44 ± 1.42	17.72 ± 0.61*	17.89 ± 0.81	18.93 ± 0.77*
1	15.98 ± 1.42	17.33 ± 0.61	17.13 ± 0.81	17.45 ± 0.77
2	18.75 ± 1.56	15.71± 0.67	16.49 ± 0.89	15.73 ± 0.85*
3	14.61 ± 1.56	11.37 ± 0.67*	17.29 ± 0.89	14.73 ± 0.85*
Female				
0	12.62 ± 0.50	11.99 ± 0.59*	11.37 ± 0.52	11.04 ± 0.47*
1	13.24 ± 0.50	10.84 ± 0.59	9.01 ± 0.52*	11.69 ± 0.47
2	12.04 ± 0.50	8.08 ± 0.59*	9.81 ± 0.52	10.46 ± 0.47
3	11.44 ± 0.71	8.07 ± 0.84*	11.12 ± 0.74	7.51 ± 0.67*
Metabolism Study				
Male				
0	13.36 ± 0.79	14.05 ± 0.94	14.35 ± 0.96	16.19 ± 1.53
2	18.49 ± 0.79	16.86 ± 0.94	15.35 ± 0.96	20.74 ± 1.53
Female				
0	12.53 ± 0.77	9.00 ± 0.90	11.61 ± 0.71	13.86 ± 1.03
2	13.36 ± 0.79	14.05 ± 0.94	14.35 ± 0.96	16.19 ± 1.53

[a] Feed consumption is given as mean ± standard error of the mean and is expressed as grams per animal per day.
* Signifies values that are significantly different ($P \leq 0.05$) from the control group by Dunnett's tests and significant ($P \leq 0.05$) linear dose trend effects based on contrast comparisons for the control group.

TABLE I3
Feed Consumption of Mice in the 14-Day Drinking Water Study of Aloe vera Extracts

Aloe vera Extract and Concentration (%)	Mean Feed Consumption[a]		
	Week 0	Week 1	Week 2
Male			
Gel			
0	3.21 ± 1.68	4.14 ± 0.22	4.42 ± 0.30
0.5	6.52 ± 1.68	4.12 ± 0.22	4.07 ± 0.30
1	4.37 ± 1.68	4.24 ± 0.22	4.77 ± 0.30
1.5	4.26 ± 1.68	4.58 ± 0.22	4.15 ± 0.30
2	7.04 ± 1.68	3.96 ± 0.22	3.88 ± 0.30
3	3.10 ± 1.68	3.94 ± 0.22	4.07 ± 0.30
Decolorized Whole Leaf			
0	3.05 ± 1.88	3.68 ± 0.45	4.46 ± 0.46
0.5	3.03 ± 1.88	4.19 ± 0.45	3.94 ± 0.46
1	2.67 ± 1.88	4.56 ± 0.45	3.81 ± 0.46
1.5	2.53 ± 1.88	3.60 ± 0.45	5.30 ± 0.46
2	2.32 ± 1.88	4.08 ± 0.45	3.70 ± 0.46
3	4.72 ± 2.66	4.35 ± 0.45	4.29 ± 0.46
Whole Leaf			
0	2.06 ± 1.33	4.48 ± 0.70	4.87 ± 0.44
0.5	2.34 ± 1.33	5.14 ± 0.70	4.33 ± 0.44
1	2.15 ± 1.33	4.44 ± 0.70	4.27 ± 0.44
1.5	2.01 ± 1.33	5.63 ± 0.70	4.95 ± 0.44
2	3.01 ± 1.33	4.53 ± 0.70	4.60 ± 0.44
3	2.05 ± 1.33	3.97 ± 0.70	4.16 ± 0.44
Female			
Gel			
0	3.85 ± 1.25	3.78 ± 0.83	4.07 ± 0.39
0.5	3.04 ± 1.25	3.68 ± 0.83	4.42 ± 0.39
1	3.15 ± 1.25	3.51 ± 0.83	4.16 ± 0.39
1.5	3.32 ± 1.25	4.00 ± 0.83	4.53 ± 0.39
2	3.34 ± 1.25	3.91 ± 0.83	4.08 ± 0.39
3	5.53 ± 1.25	5.35 ± 0.83	3.91 ± 0.39
Decolorized Whole Leaf			
0	4.33[b]	3.64 ± 0.78	4.61 ± 0.60
0.5	3.61[b]	4.13 ± 0.78	3.96 ± 0.60
1	4.10[b]	3.76 ± 0.78	4.15 ± 0.60
1.5	4.09[b]	3.35 ± 0.78	3.92 ± 0.60
2	4.01[b]	4.06 ± 0.78	4.07 ± 0.60
3	3.83[b]	4.68 ± 0.78	4.62 ± 0.60
Whole Leaf			
0	2.22 ± 1.21	4.14 ± 1.18	5.10 ± 1.08
0.5	3.15 ± 1.21	4.97 ± 1.18	4.15 ± 1.08
1	3.03 ± 1.21	4.80 ± 1.18	5.12 ± 1.08
1.5	1.94 ± 1.21	4.67 ± 1.18	5.12 ± 1.08
2	2.14 ± 1.21	3.78 ± 1.18	4.16 ± 1.08
3	2.09 ± 1.21	4.35 ± 1.18	4.21 ± 1.08

[a] Feed consumption is given as mean ± standard error of the mean and is expressed as grams per animal per day.
[b] Due to technician error, mean feed consumption values represent the consumption of one cage and error could not be determined.

TABLE I4
Feed Consumption of Mice in the 13-Week Drinking Water Study of Aloe vera Whole Leaf Extract

Concentration (%)	Mean Feed Consumption[a]			
	Week 0	Week 4	Week 8	Week 13
Subchronic Study				
Male				
0	7.19 ± 1.16	6.45 ± 0.51	5.51 ± 0.44	6.03 ± 0.33
1	5.96 ± 1.16	6.44 ± 0.51	5.87 ± 0.44	6.28 ± 0.33
2	4.84 ± 1.16	7.32 ± 0.51	6.92 ± 0.44	7.27 ± 0.33
3	7.92 ± 1.16	6.99 ± 0.51	5.76 ± 0.44	6.33 ± 0.33
Female				
0	3.82 ± 0.26	4.31 ± 0.18*	4.96 ± 0.42	4.66 ± 0.23
1	4.07 ± 0.26	4.68 ± 0.18	5.29 ± 0.42	5.24 ± 0.23
2	3.24 ± 0.26	4.74 ± 0.18	5.03 ± 0.42	5.33 ± 0.23
3	4.10 ± 0.26	4.92 ± 0.18	4.77 ± 0.42	4.62 ± 0.23
Metabolism Study				
Male				
0	4.84 ± 0.12	4.83 ± 0.24	5.79 ± 0.62	4.65 ± 0.22
2	4.76 ± 0.12	4.58 ± 0.24	5.02 ± 0.62	4.43 ± 0.22
Female				
0	3.96 ± 0.29	5.15 ± 0.06	5.43 ± 0.22	4.17 ± 0.24
2	4.15 ± 0.29	4.59 ± 0.06	5.09 ± 0.22	4.24 ± 0.24

[a] Feed consumption is given as mean ± standard error of the mean and is expressed as grams per animal per day.
* Significant ($P \leq 0.05$) linear dose trend effects are based on contrast comparisons for the control group.

APPENDIX J
WATER CONSUMPTION IN THE 2-YEAR DRINKING WATER STUDY OF ALOE VERA WHOLE LEAF EXTRACT

TABLE J1
Water Consumption by Male Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

Week	0.0%		0.5%		1.0%		1.5%	
	Water (g/day)[a]	Body Weight (g)[b]	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)
4	24.56	214.9	25.12	211.3	28.80	200.0	32.45	183.8
8	24.98	290.7	25.95	286.9	32.18	272.5	37.78	252.3
12	23.92	339.5	23.12	338.7	28.22	321.8	34.82	296.5
16	22.39	375.3	21.50	373.9	26.68	356.8	30.65	331.1
20	20.39	401.4	20.38	399.8	25.56	383.5	29.62	357.1
24	20.31	426.7	19.47	421.5	24.82	405.6	29.32	379.5
28	18.80	447.7	19.47	440.8	23.54	424.2	28.51	398.9
32	18.27	463.6	19.71	457.6	24.11	438.0	29.10	415.2
36	18.00	476.0	19.25	471.4	24.75	450.1	29.57	426.4
40	17.41	486.3	19.57	480.8	25.09	459.3	30.26	436.0
44	17.17	494.2	19.10	487.9	24.61	465.7	29.03	442.2
48	17.03	503.0	18.78	495.9	24.79	473.2	29.57	448.6
52	17.07	509.7	19.01	504.6	24.99	479.9	29.97	453.8
56	17.47	514.0	19.21	508.7	24.35	484.3	30.75	458.8
60	17.82	520.7	19.58	513.5	23.71	490.1	34.07	462.4
64	18.35	524.2	19.78	516.9	23.90	493.6	33.75	464.5
68	19.01	525.5	20.29	516.8	24.30	494.1	32.35	464.6
72	19.73	525.1	21.12	516.9	24.16	492.2	28.50	466.1
76	20.82	523.0	22.18	512.0	26.52	491.0	29.73	464.5
80	22.46	520.2	24.89	509.1	28.29	492.5	30.83	463.0
84	23.88	515.4	25.15	494.8	28.73	489.1	31.03	460.6
88	25.05	497.1	23.11	488.5	27.90	482.5	29.96	458.0
92	27.35	486.7	25.76	477.6	28.41	473.2	29.64	454.7
96	28.07	461.5	28.62	469.8	31.65	467.2	30.75	448.1
100	31.30	443.8	30.18	450.9	35.18	448.4	31.73	434.9
104	34.30	409.9	32.63	410.3	36.10	423.8	32.22	408.7
Mean for weeks								
4-104	21.76	457.5	22.48	452.2	26.98	436.6	31.0	412.7

[a] Mean values are daily water consumptions in grams per animal per cage by week on study.
[b] Mean body weight in grams per animal.

TABLE J2
Water Consumption by Female Rats
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

Week	0.0%		0.5%		1.0%		1.5%	
	Water (g/day)[a]	Body Weight (g)[b]	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)
4	20.05	149.7	19.70	147.9	22.46	142.6	23.01	128.9
8	20.41	179.3	20.40	176.3	22.52	171.8	24.45	160.7
12	19.02	196.8	18.17	197.1	17.97	192.2	18.53	182.2
16	18.15	209.1	17.41	209.7	17.17	204.6	16.73	193.0
20	17.23	220.3	16.35	219.3	16.34	213.9	16.50	201.6
24	17.18	228.9	16.08	230.0	16.43	221.5	15.96	208.8
28	16.82	238.9	15.97	238.8	15.92	231.2	14.98	216.4
32	16.70	247.3	15.94	248.1	15.91	238.9	15.99	221.9
36	17.07	255.7	16.12	255.7	16.91	244.0	17.54	227.2
40	16.01	261.0	16.49	261.3	17.64	250.6	18.82	231.5
44	15.74	267.0	16.04	268.1	17.23	255.9	17.77	237.1
48	15.99	273.5	15.61	273.6	17.23	263.0	17.41	241.7
52	15.88	280.1	15.54	280.8	16.90	268.9	17.54	244.7
56	16.08	287.0	16.14	286.8	17.00	273.7	19.34	248.6
60	16.68	295.5	16.63	296.1	16.68	280.5	23.36	254.0
64	16.99	302.6	16.54	305.6	16.87	286.0	23.42	259.4
68	17.43	308.6	17.10	312.3	17.73	291.9	23.03	262.3
72	18.13	315.4	17.36	319.9	18.50	294.6	20.85	264.8
76	18.29	322.3	18.09	322.6	20.33	298.9	20.72	268.6
80	18.68	327.0	19.97	327.7	22.30	306.3	22.45	274.4
84	18.64	334.1	20.38	334.1	21.99	307.9	22.20	279.5
88	18.69	336.9	20.26	338.6	22.00	308.5	21.42	281.3
92	19.26	341.4	20.70	340.5	22.57	312.7	21.90	283.2
96	19.99	343.5	21.99	339.6	22.68	310.0	24.19	282.6
100	19.68	339.8	23.37	334.4	23.39	304.0	25.38	280.7
104	21.27	333.6	23.93	321.3	23.46	288.3	25.70	265.2
Mean for weeks								
4-104	17.92	276.7	18.16	276.4	19.08	260.1	20.35	238.5

[a] Mean values are daily water consumptions in grams per animal per cage by week on study.
[b] Mean body weight in grams per animal.

TABLE J3
Water Consumption by Male Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

Week	0%		1%		2%		3%	
	Water (g/day)[a]	Body Weight (g)[b]	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)
4	6.47	29.1	8.33	28.9	10.05	29.3	11.34	28.7
8	7.47	33.0	9.95	32.3	12.02	32.9	13.22	31.9
12	8.61	35.4	10.98	34.6	13.87	34.7	15.36	34.0
16	8.48	37.5	11.97	35.6	14.15	36.1	17.63	36.6
20	7.80	39.6	11.62	37.5	13.95	37.8	15.92	37.5
24	8.54	41.0	12.51	38.5	14.18	39.3	15.84	39.0
28	7.57	43.0	11.23	40.3	12.23	40.4	15.4	40.1
32	7.50	43.5	12.13	42.0	13.12	41.6	14.93	41.7
36	6.65	45.2	11.38	42.4	13.01	42.6	13.67	42.6
40	6.96	45.6	12.37	43.5	12.94	43.0	14.51	43.0
44	7.04	46.5	11.62	43.6	13.26	43.8	13.89	43.4
48	7.24	46.7	12.21	44.4	14.22	43.9	14.55	44.4
52	7.18	46.8	13.10	44.8	13.84	44.3	15.65	44.0
56	7.54	47.1	10.59	45.2	14.56	44.7	15.84	44.7
60	7.05	47.7	11.27	45.7	13.53	45.3	14.81	44.7
64	7.73	47.5	12.37	45.3	14.46	45.0	15.22	44.1
68	8.36	47.1	13.80	44.8	14.72	44.2	14.92	43.4
72	8.13	47.0	12.62	44.0	15.08	44.4	15.67	42.7
76	8.03	46.1	12.39	42.9	15.86	42.9	16.53	41.6
80	8.45	45.6	14.24	42.0	17.87	41.6	17.41	40.4
84	8.83	45.2	14.39	41.9	15.54	40.9	17.21	40.9
88	8.16	44.9	13.58	42.0	16.62	41.7	16.53	41.3
92	7.88	44.5	11.79	41.2	14.54	40.9	19.18	40.8
96	7.94	43.9	12.98	40.7	14.72	40.1	19.62	40.0
100	8.85	42.7	12.28	40.3	16.18	39.7	19.16	38.9
104	8.49	41.5	11.20	39.1	13.27	38.2	16.70	37.6
Mean for weeks								
4-104	7.81	43.2	12.03	40.9	14.15	40.7	15.80	40.3

[a] Mean values are daily water consumptions in grams per animal per cage by week on study.
[b] Mean body weight in grams per animal.

TABLE J4
Water Consumption by Female Mice
in the 2-Year Drinking Water Study of Aloe vera Whole Leaf Extract

Week	0%		1%		2%		3%	
	Water (g/day)[a]	Body Weight (g)[b]	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)
4	5.11	21.9	6.26	22.4	7.31	22.1	9.51	22.3
8	5.01	24.0	6.26	24.5	8.01	24.4	11.31	24.5
12	5.02	25.7	6.35	25.9	9.63	26.0	12.14	26.1
16	4.82	26.4	6.17	27.8	9.21	26.8	12.25	26.6
20	4.90	27.9	6.69	28.4	9.49	28.2	11.46	27.5
24	5.12	29.4	6.82	30.2	9.39	29.8	12.12	29.3
28	5.07	30.9	6.77	31.6	9.41	31.2	11.69	30.6
32	5.56	31.9	7.99	32.3	10.62	31.8	12.29	31.3
36	4.95	32.6	7.16	34.8	10.31	33.6	12.02	32.6
40	5.24	34.1	8.32	35.0	10.94	34.6	12.80	33.1
44	5.14	35.0	7.70	36.2	11.64	36.1	12.73	34.3
48	5.38	36.4	8.70	37.2	12.09	37.4	13.70	35.5
52	5.55	37.0	9.36	38.0	12.84	37.4	14.99	36.0
56	5.25	38.5	8.24	39.5	12.62	38.8	14.93	37.2
60	5.73	39.7	8.00	41.4	12.38	40.3	14.57	38.3
64	5.11	40.6	8.07	42.2	12.70	41.1	14.51	38.9
68	5.96	41.4	9.16	44.0	12.91	42.9	14.98	40.5
72	5.53	43.4	9.36	45.0	13.56	44.4	15.12	41.7
76	5.69	43.7	9.05	44.7	13.21	44.1	15.10	41.6
80	5.92	43.6	10.16	45.3	14.38	45.1	16.20	42.3
84	5.42	44.1	10.00	45.3	13.09	45.7	16.37	42.5
88	5.75	45.2	9.52	45.9	12.34	46.3	17.00	42.7
92	5.27	45.9	9.29	46.9	13.76	45.7	17.31	44.0
96	5.24	45.2	10.27	47.0	15.18	46.1	17.53	43.4
100	6.26	45.0	10.19	46.5	15.84	45.9	18.83	43.7
104	5.80	45.5	8.73	46.4	12.25	46.3	14.61	42.2
Mean for weeks								
4-104	5.38	36.7	8.25	37.9	11.74	37.4	14.08	35.7

[a] Mean values are daily water consumptions in grams per animal per cage by week on study.
[b] Mean body weight in grams per animal.

APPENDIX K
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-31 RAT AND MOUSE RATION

TABLE K1
Ingredients of NIH-31 Rat and Mouse Diet[a]

Ingredients	Percent by Weight
Ground whole hard wheat	35.5
Ground #2 yellow shelled corn	21.0
Ground whole oats	10.0
Wheat middlings	10.0
Fish meal (60% protein)	9.0
Soybean meal (48.5% protein)	5.0
Alfalfa meal (17% protein)	2.0
Corn gluten meal (60%)	2.0
Dicalcium phosphate[b]	1.5
Soy oil	1.5
Brewers dried yeast	1.0
Ground limestone[b]	0.5
Premixes	0.5
Salt	0.5

[a] Ingredients ground to pass through a U.S. Standard Screen No. 16 before mixing.
[b] Specific ingredient requirement for cadmium content not to exceed 1 mg/kg.

TABLE K2
Vitamins and Minerals in NIH-31 Rat and Mouse Diet[a]

	Amount	Source
Vitamins		
A	22,000,000 IU	Vitamin A palmitate or acetate
D_3	3,800,000 IU	D-activated animal sterol
K_3	20 g	Menadione activity
Choline	700 g	Choline chloride
dl-α-Tocopheryl acetate	15 g	
Folic acid	1 g	
Niacin	20 g	
d-Pantothenic acid	25 g	*d*-Calcium pantothenate
Riboflavin	5 g	
Thiamine	65 g	Thiamine mononitrate
B_{12}	14 g	
Pyridoxine	2 g	Pyridoxine hydrochloride
Biotin	0.12 g	*d*-Biotin
Minerals		
Magnesium	400 g	Magnesium oxide
Manganese	100 g	Manganese oxide
Iron	60 g	Iron sulfate
Zinc	10 g	Zinc oxide
Copper	4 g	Copper sulfate
Iodine	1.5 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2000 lb) of finished product

TABLE K3
Nutrient Composition of NIH-31 Rat and Mouse Diet

Nutrient	Mean ± S.D.	Number of Samples
Crude protein (% by weight)	19.0 ± 0.6	27
Crude fat (% by weight)	5.95 ± 0.73	27
Volatiles (% by weight)	7.92 ± 0.63	27
Vitamins		
A (μg/g)	11.7 ± 1.6	27
E (μg/g)	58.1 ± 12.2	27
B_1 (mg/g)	0.088 ± 0.009	27
Minerals		
Selenium (μg/g)	0.37 ± 0.11	27

TABLE K4
Contaminant Levels in NIH-31 Rat and Mouse Diet

	Mean ± S.D.	Number of Positive Samples / Number of Samples Tested
Arsenic (μg/g)	0.05 ± 0.06	13/27
Cadmium (μg/g)	0.05 ± 0.12	5/27
Lead (μg/g)	0.43 ± 0.11	24/27
Aflatoxin B1 (ppb)	<mdl	0/27
Aflatoxin B2 (ppb)	<mdl	0/27
Aflatoxin G1 (ppb)	<mdl	0/27
Aflatoxin G2 (ppb)	<mdl	0/27
Total Fumonisin	288 ± 183	27/27

APPENDIX L
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Blood from each sentinel animal was collected, allowed to clot and the serum was separated. The serum was analyzed by Multiplex Fluorescent Immunoassay (MFI) for the presence of specific antibodies by the Research Animal Diagnostic Laboratory, University of Missouri, Columbia, Missouri. The laboratory serology method and viral/mycoplasma agent for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
MICE	
MFI	
Mouse Hepatitis Virus (MHV)	6, 13, 19, and 25 months
Sendai	6, 13, 19, and 25 months
Pneumonia Virus of Mice (PVM)	6, 13, 19, and 25 months
Reovirus Type 3 (REO3)	6, 13, 19, and 25 months
Theiler's Murine Encephalomyelitis Virus (TMEV GDVII)	6, 13, 19, and 25 months
Ectromelia	6, 13, 19, and 25 months
Polyoma	6, 13, 19, and 25 months
Mycoplasma pulmonis	6, 13, 19, and 25 months
Minute Virus of Mice (MMV)	6, 13, 19, and 25 months
Mouse Parvovirus (MPV)	6, 13, 19, and 25 months
Parvo NS-1	6, 13, 19, and 25 months
Epizootic Diarrhea of Infant Mice Virus (EDIM)	6, 13, 19, and 25 months
Lymphocytic Choriomeningitis Virus (LCM)	6, 13, 19, and 25 months
Polymerase Chain Reaction (PCR)	
Helicobacter hepaticus	
RATS	
MFI	
Rat Coronavirus/Sialodacryoadenitis (RCV/SDAV)	6, 12, 18, and 25 months
Sendai	6, 12, 18, and 25 months
Pneumonia Virus of Mice (PVM)	6, 12, 18, and 25 months
TMEV GDVII	6, 12, 18, and 25 months
Mycoplasma pulmonis	6, 12, 18, and 25 months
Parvo NS-1	6, 12, 18, and 25 months
Routine Culturing Procedures	
Pasteurella pneumotropica	

RESULTS

All serology test results were negative.

Helicobacter hepaticus was detected via PCR in five of the sentinel mice. *Pasteurella pneumotropica* was detected in one of the sentinel rats.